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Trying 31060000009999...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS? ### Status: Signing onto Dialog ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS?  ********
Welcome to DIALOG

### Status: Login successfulDialog level 05.16.01D

Last logoff:  13feb07 13:18:28
Logon file405 16feb07 15:30:20
           *** ANNOUNCEMENTS ***
                  ***
NEW FILES RELEASED
***Engineering Index Backfile (File 988)
***EMCare (File 45)
***Trademarkscan - South Korea (File 655)

RESUMED UPDATING
***File 141, Reader's Guide Abstracts
                  ***
RELOADS COMPLETED
***Files 340, 341 & 942, CLAIMS/U.S. Patents - 2006 reload now online
***Files 173 & 973, Adis Clinical Trials Insight
                              ***
DATABASES REMOVED

Chemical Structure Searching now available in Prous Science Drug
Data Report (F452), Prous Science Drugs of the Future (F453),
IMS R&D Focus (F445/955), Pharmaprojects (F128/928), Beilstein
Facts (F390), Derwent Chemistry Resource (F355) and Index Chemicus
(File 302).
                  ***
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* * *
SYSTEM:HOME
Cost is in DialUnits
Menu System II: D2 version 1.8.0 term=ASCII
                     *** DIALOG HOMEBASE(SM) Main Menu ***

 Information:
  1.  Announcements (new files, reloads, etc.)
  2.  Database, Rates, & Command Descriptions
  3.  Help in Choosing Databases for Your Topic
  4.  Customer Services (telephone assistance, training, seminars, etc.)
  5.  Product Descriptions

 Connections:
  6.  DIALOG(R) Document Delivery
  7.  Data Star(R)

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      /H = Help            /L = Logoff          /NOMENU = Command Mode


Enter an option number to view information or to connect to an online
 service.  Enter a BEGIN command plus a file number to search a database
(e.g., B1 for ERIC).
?
Terminal set to DLINK
                    *** DIALOG HOMEBASE(SM) Main Menu ***

 Information:
  1.  Announcements (new files, reloads, etc.)
  2.  Database, Rates, & Command Descriptions
  3.  Help in Choosing Databases for Your Topic
  4.  Customer Services (telephone assistance, training, seminars, etc.)
  5.  Product Descriptions

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  6.  DIALOG(R) Document Delivery
  7.  Data Star(R)

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      /H = Help            /L = Logoff          /NOMENU = Command Mode


Enter an option number to view information or to connect to an online
 service.  Enter a BEGIN command plus a file number to search a database
(e.g., B1 for ERIC).
? b biosci

>>>          44 is unauthorized
>>>          76 is unauthorized
>>>2 of the specified files are not available
       16feb07 15:30:24 User276653 Session D83.1
            $0.00    0.245 DialUnits FileHomeBase
     $0.00  Estimated cost FileHomeBase
     $0.02  TELNET
     $0.02  Estimated cost this search
     $0.02  Estimated total session cost   0.245 DialUnits


SYSTEM:OS  - DIALOG OneSearch
  File   5:Biosis Previews(R) 1969-2007/Feb W2
         (c) 2007 The Thomson Corporation
*File   5: In preparation for coming enhancements, accession numbers will
change soon. See HELP NEWS 5 for details.
  File  24:CSA Life Sciences Abstracts 1966-2007/Nov
         (c) 2007 CSA.
  File  28:Oceanic Abstracts 1966-2007/Nov
         (c) 2007 CSA.
  File  34:SciSearch(R) Cited Ref Sci 1990-2007/Feb W2
         (c) 2007 The Thomson Corp
  File  35:Dissertation Abs Online 1861-2007/Jan
         (c) 2007 ProQuest Info&Learning
  File  40:Enviroline(R) 1975-2007/Dec
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(c) 2007 Congressional Information Service
File 41:Pollution Abstracts 1966-2007/Nov
(c) 2007 CSA.
File 45:EMCare 2007/Feb W2
(c) 2007 Elsevier B.V.
File 50:CAB Abstracts 1972-2007/Jan
(c) 2007 CAB International
File 65:Inside Conferences 1993-2007/Feb 16
(c) 2007 BLDSC all rts. reserv.
File 71:ELSEVIER BIOBASE 1994-2007/Feb W2
(c) 2007 Elsevier B.V.
File 73:EMBASE 1974-2007/Feb 16
(c) 2007 Elsevier B.V.
File 91:MANTIS(TM) 1880-2006/Jan
2001 (c) Action Potential
File 94:JICST-EPlus 1985-2007/Feb W3
(c)2007 Japan Science and Tech Corp(JST)
***File 94: UD200609W2 is the last update for 2006. UD200701W1 is the** first update for 2007. The file is complete and up to date.
File 98:General Sci Abs 1984-2007/Feb
(c) 2007 The HW Wilson Co.
File 110:WasteInfo 1974-2002/Jul
(c) 2002 AEA Techn Env.
***File 110: This file is closed (no updates)**
File 135:NewsRx Weekly Reports 1995-2007/Feb W2
(c) 2007 NewsRx
File 136:BioEngineering Abstracts 1966-2007/Nov
(c) 2007 CSA.
File 143:Biol. & Agric. Index 1983-2007/Jan
(c) 2007 The HW Wilson Co
File 144:Pascal 1973-2007/Feb W1
(c) 2007 INIST/CNRS
File 155:MEDLINE(R) 1950-2007/Feb 13
(c) format only 2007 Dialog
***File 155: MEDLINE has resumed updating with UD20061209. Please** see HELP NEWS 154 for details.
File 164:Allied & Complementary Medicine 1984-2007/Feb
(c) 2007 BLHCIS
File 172:EMBASE Alert 2007/Feb 16
(c) 2007 Elsevier B.V.
File 185:Zoological Record Online(R) 1978-2007/Feb
(c) 2007 The Thomson Corp.
File 357:Derwent Biotech Res. _1982-2007/Feb W2
(c) 2007 The Thomson Corp.
File 369:New Scientist 1994-2007/Oct W5
(c) 2007 Reed Business Information Ltd.
File 370:Science 1996-1999/Jul W3
(c) 1999 AAAS
***File 370: This file is closed (no updates). Use File 47 for more current** information.
File 391:Beilstein Reactions 2006/Q4
(c) 2006 Beilstein GmbH
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
(c) 2006 The Thomson Corp
File 467:ExtraMED(tm) 2000/Dec
(c) 2001 Informania Ltd.

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      Set   Items   Description
      ---   -----   -----------
? s chaperone
      S1    59277   CHAPERONE
? s horse(w)radish(w)peroxidase
           199499   HORSE
            25883   RADISH
           379989   PEROXIDASE
      S2     2585   HORSE(W)RADISH(W)PEROXIDASE
? s s1 and s2
           59277   S1
            2585   S2
      S3        1   S1 AND S2
? t s3/9,k/1
```

3/9,K/1 (Item 1 from file: 144)

14965851 PASCAL No.: 01-0118910

Improvement of productivity of active horseradish peroxidase in Escherichia coli by coexpression of Dsb proteins

KONDO Akihiko; KOHDA Jiro; ENDO Yasunori; SHIROMIZU Tokuhisa; KUROKAWA Yoichi; NISHIHARA Kazuyo; YANAGI Hideki; YURA Takashi; FUKUDA Hideki

Department of Chemical Science and Engineering, Faculty of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan; Division of Molecular Science, Graduate School of Science and Technology, SUP 2 Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan; HSP Research Institute, Kyoto Research Park, Kyoto 600-8813, Japan

Journal: Journal of bioscience and bioengineering, 2000, 90 (6) 600-606
ISSN: 1389-1723 Availability: INIST-8234; 354000094103590030
No. of Refs.: 28 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Japan
Language: English

Coexpression of two classes of folding accessory proteins, molecular chaperones and foldases, can be expected to improve the productivity of soluble and active recombinant proteins. In this study, horseradish peroxidase (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in Escherichia coli. The effects of coexpression of a series of folding accessory proteins (DnaK, DnaJ, GrpE, GroEL/ES, trigger factor (TF), DsbA, DsbB, DsbC, DsbD, and thioredoxin (Trx)) on the productivity of active HRP in E. coli were examined. Active HRP was produced by very mild induction with 1 mu M isopropyl- beta -D-thiogalactopyranoside (IPTG) at 37 Degree C, whereas the amount of active HRP produced by the induction with 1 mM IPTG was negligibly small. Active HRP production was increased significantly by coexpression of DsbA-DsbB (DsbAB) or DsbC-DsbD (DsbCD), while coexpression of molecular chaperones did not improve active HRP production. The growth of E. coli cells was inhibited significantly by the induction with 1 mM IPTG in a HRP single expression system. In contrast, when HRP was coexpressed with DsbCD, the growth inhibition of E. coli was not observed. Therefore, coexpression of Dsb proteins improves both the cell growth and the productivity of HRP.

English Descriptors: Production; Escherichia coli; **Horse radish** ; **Peroxidase** ; Refolding; **Chaperone** ; Gene; Microorganism culture; Recombinant protein; Gene expression; Gene coexpression

Broad Descriptors: Enterobacteriaceae; Bacteria; Peroxidases; Oxidoreductases; Enzyme; Enterobacteriaceae; Bacterie; Peroxidases; Oxidoreductases; Enzyme; Enterobacteriaceae; Bacteria; Peroxidases; Oxidoreductases; Enzima
French Descriptors: Production; Escherichia coli; Raifort; Peroxidase; Repliement; Chaperon; Gene; Culture microorganisme; Proteine recombinante ; Expression genique; Coexpression genique

Classification Codes: 002A31C02A8; 215

English Descriptors: Production; Escherichia coli; **Horse radish** ; **Peroxidase** ; Refolding; **Chaperone** ; Gene; Microorganism culture; Recombinant protein; Gene expression; Gene coexpression
? s s1 and HRP
59277 S1
51633 HRP
S4 128 S1 AND HRP
? t s4/9,k/1-10

4/9,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

19218364 BIOSIS NO.: 200600563759
WtsE, an AvrE-family effector protein from Pantoea stewartii subsp stewartii, causes disease-associated cell death in corn and requires a chaperone **protein for stability**
AUTHOR: Ham Jong Hyun; Majerczak Doris R; Arroyo-Rodriguez Angel S; Mackey David M; Coplin David L (Reprint)
AUTHOR ADDRESS: Ohio State Univ, Dept Plant Pathol, Columbus, OH 43210 USA **USA
AUTHOR E-MAIL ADDRESS: coplin.3@osu.edu
JOURNAL: Molecular Plant-Microbe Interactions 19 (10): p1092-1102 OCT 2006 2006
ISSN: 0894-0282
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The pathogenicity of Pantoea stewartii subsp. stewartii to sweet corn and maize requires a **Hrp** type III secretion system. In this study, we genetically and functionally characterized a disease-specific (Dsp) effector locus, composed of wtsE and wtsF, that is adjacent to the **hrp** gene cluster. WtsE, a member of the AvrE family of effector proteins, was essential for pathogenesis on corn and was complemented by DspA/E from Erwinia amylovora. An intact C-terminus of WtsE, which contained a putative endoplasmic reticulum membrane retention signal, was important for function of WtsE. Delivery of WtsE into sweet corn leaves by an Escherichia coli strain carrying the **hrp** cluster of Erwinia chrysanthemi caused water-soaking and necrosis. WtsE-induced cell death was not inhibited by cycloheximide treatment, unlike the hypersensitive response caused by a known Avr protein, AvrRxo1. WtsF, the putative **chaperone** of WtsE, was not required for secretion of WtsE from P stewartii, and the virulence of wtsF mutants was reduced only at low

inoculum concentrations. However, WtsF was required for full accumulation of WtsE within the bacteria at low temperatures. In contrast, WtsF was needed for efficient delivery of WtsE from E. coli via the Erwinia chrysanthemi **Hrp** system.

REGISTRY NUMBERS: 66-81-9: cycloheximide
DESCRIPTORS:
MAJOR CONCEPTS: Pharmacology; Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Gramineae--Monocotyledones, Angiospermae, Spermatophyta, Plantae
ORGANISMS: Escherichia coli (Enterobacteriaceae); Erwinia chrysanthemi (Enterobacteriaceae); Erwinia amylovora (Enterobacteriaceae); Pantoea stewartii stewartii (Enterobacteriaceae); maize (Gramineae); corn (Gramineae)
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Angiosperms ; Monocots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: cycloheximide--enzyme inhibitor-drug, antifungal-drug, antiinfective-drug
GENE NAME: Erwinia amylovora **hrp** gene (Enterobacteriaceae) {Erwinia amylovora hyperpolarizing receptor potential gene}
MISCELLANEOUS TERMS: hypertensive response
CONCEPT CODES:
03502 Genetics - General
03504 Genetics - Plant
10060 Biochemistry studies - General
12512 Pathology - Therapy
22002 Pharmacology - General
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
38502 Chemotherapy - General, methods and metabolism
38508 Chemotherapy - Antifungal agents
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae
25305 Gramineae

...from Pantoea stewartii subsp stewartii, causes disease-associated cell death in corn and requires a chaperone **protein for stability**

ABSTRACT: The pathogenicity of Pantoea stewartii subsp. stewartii to sweet corn and maize requires a **Hrp** type III secretion system. In this study, we genetically and functionally characterized a disease-specific (Dsp) effector locus, composed of wtsE and wtsF, that is adjacent to the **hrp** gene cluster. WtsE, a member of the AvrE family of effector proteins, was essential for...

...WtsE. Delivery of WtsE into sweet corn leaves by an Escherichia coli strain carrying the **hrp** cluster of Erwinia chrysanthemi caused water-soaking and necrosis. WtsE-induced cell death was not...

...treatment, unlike the hypersensitive response caused by a known Avr protein, AvrRxo1. WtsF, the putative **chaperone** of WtsE, was not required for secretion of WtsE from P stewartii, and the virulence...

...WtsF was needed for efficient delivery of WtsE from E. coli via the Erwinia chrysanthemi **Hrp** system.

DESCRIPTORS:
  GENE NAME: Erwinia amylovora **hrp** gene (Enterobacteriaceae) {Erwinia amylovora hyperpolarizing receptor potential gene}

4/9,K/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18912908 BIOSIS NO.: 200600258303
Functional specificity of the mammalian Beclin-Vps34 Pl 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking
AUTHOR: Zeng Xuehuo; Overmeyer Jean H; Maltese William A (Reprint)
AUTHOR ADDRESS: Med Univ Ohio, Dept Biochem and Canc Biol, Toledo, OH 43614 USA**USA
AUTHOR E-MAIL ADDRESS: wmaltese@meduchio.edu
JOURNAL: Journal of Cell Science 119 (2): p259-270 JAN 15 2006 2006
ISSN: 0021-9533
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Beclin 1 was originally identified as a novel Bcl-2-interacting protein, but co-immunoprecipitation studies suggest that the major physiological partner for Beclin 1 is the mammalian class III phosphatidylinositol 3-kinase (PI 3-kinase) Vps34. Beclin I has been proposed to function as a tumor suppressor by promoting cellular macroautophagy, a process that is known to depend on Vps34. However, an alternative role for Beclin I in modulating normal Vps34-dependent protein trafficking pathways has not been ruled out. This possibility was examined in U-251 glioblastoma cells. Immunoprecipitates of endogenous Beclin I contained human Vps34 (hVps34), but not Bcl-2. Suppression of Beclin 1 expression by short interfering (si)RNA-mediated gene silencing blunted the autophagic response of the cells to nutrient deprivation or C-2-ceramide. However, other PI 3-kinase-dependent trafficking pathways, such as the post-endocytic sorting of the epidermal growth factor receptor (EGFR) or the proteolytic processing of procathepsin D en route from the trans-Golgi network (TGN) to lysosomes, were not affected. Depletion of Beclin I did not reduce endocytic internalization of a fluid phase marker (horseradish peroxidase, **HRP**) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin I functions mainly to engage hVps34 in the autophagic pathway.

REGISTRY NUMBERS: 9003-99-0: horseradish peroxidase; 86921-29-1: procathepsin D
ENZYME COMMISSION NUMBER: EC 1.11.1.7: horseradish peroxidase; EC 2.7.1.137 : Vps34p
DESCRIPTORS:
  MAJOR CONCEPTS: Enzymology--Biochemistry and Molecular Biophysics; Membranes--Cell Biology
  BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
  ORGANISMS: U-251 cell line (Hominidae)--human glioblastoma cells
  COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates;

Vertebrates
CHEMICALS & BIOCHEMICALS: Bcl-2; epidermal growth factor receptor; horseradish peroxidase; Vps34p; procathepsin D; beclin-1
MISCELLANEOUS TERMS: endocytosis; vesicular trafficking; cellular macroautophagy
CONCEPT CODES:
02508 Cytology - Human
10064 Biochemistry studies - Proteins, peptides and amino acids
10508 Biophysics - Membrane phenomena
10802 Enzymes - General and comparative studies: coenzymes
17002 Endocrine - General
BIOSYSTEMATIC CODES:
86215 Hominidae

...ABSTRACT: of Beclin I did not reduce endocytic internalization of a fluid phase marker (horseradish peroxidase, **HRP**) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin...

4/9,K/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18421537 BIOSIS NO.: 200510116037
A chaperone **-like HrpG protein acts as a suppressor of HrpV in regulation of the Pseudomonas syringae pv. syringae type III secretion system**
AUTHOR: Wei Chia-Fong; Deng Wen-Ling; Huang Hsiou-Chen (Reprint)
AUTHOR ADDRESS: Natl Chung Hsing Univ, Grad Inst Biotechnol, Taichung 40224, Taiwan**Taiwan
AUTHOR E-MAIL ADDRESS: hchuang@dragon.nchu.edu.tw
JOURNAL: Molecular Microbiology 57 (2): p520-536 JUL 05 2005
ISSN: 0950-382X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The cloned **hrp** /hrc cluster of Pseudomonas syringae pv. syringae 61 (Pss61) contains 28 proteins, and many of those are assembled into a type III secretion system (TTSS) that is responsible for eliciting the hypersensitive response (HR) in non-host plants and causing diseases on host plants (Huang et al., 1995). hrpG, the second gene in the hrpC operon, encodes a 15.4 kDa cytoplasmic protein whose predicted structure is similar to SicP (E-value: 0.19), a TTSS **chaperone** of Salmonella typhimurium. Two non-polar hrpG mutants, Pss61-N826 and Pss61-N674, were produced to investigate the biological function of hrpG gene. Pss61-N826, generated by replacing the coding sequence of hrpG with an nptII gene lacking both the promoter and the terminator, was found to be capable of eliciting the wild-type HR; whereas Pss61-N674 generated by replacement of a terminatorless nptII gene in the hrpG coding sequence showed the delayed HR phenotype. Northern and Western blotting analyses showed that the expression of hrpZ, hrcJ and hrcQb genes residing on two different operons in Pss61-N674 was reduced due to the nptII promoter-driven constitutive expression of hrpV that codes for a negative regulator. Interestingly, a plasmid-borne hrpG can derepress the **hrp** expression in

Pss61-N674 and in Pss61 overexpressing HrpV without decreasing the hrpV transcript. Moreover, results of yeast two-hybrid assay, pull-down assay and far Western analysis show that HrpG and HrpV interact with each other in vivo and in vitro. Additionally, HrpV interacts with a positive regulator HrpS according to analysis of a yeast two-hybrid system. Based on the results presented in this study, we propose that HrpG acts as a suppressor of the negative regulator HrpV mediated via protein-protein interaction, leading to modulation of **hrp** /hrc expression subsequently freeing HrpS to promote the activation of other downstream **hrp** /hrc genes.

DESCRIPTORS:
MAJOR CONCEPTS: Infection; Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Salmonella typhimurium (Enterobacteriaceae); Pseudomonas syringae (Pseudomonadaceae)--pathogen, pathovar-syringae
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
DISEASES: leaf spots--fungal disease; foliar necrosis--fungal disease
CHEMICALS & BIOCHEMICALS: plasmid; HrpG protein-- **chaperone** -like; HrpV ; SicP
GENE NAME: Pseudomonas syringae **hrp** gene cluster (Pseudomonadaceae); Pseudomonas syringae hrc gene cluster (Pseudomonadaceae); Pseudomonas syringae hrpG gene (Pseudomonadaceae)--mutant; Pseudomonas syringae hrpC gene (Pseudomonadaceae); Pseudomonas syringae nptll gene (Pseudomonadaceae); Pseudomonas syringae hrpZ gene (Pseudomonadaceae)--expression; Pseudomonas syringae hrcJ gene (Pseudomonadaceae)--expression; Pseudomonas syringae hrcQb gene (Pseudomonadaceae)--expression; Pseudomonas syringae hrpV gene (Pseudomonadaceae)--expression
METHODS & EQUIPMENT: Western blot--electrophoretic techniques, immunologic techniques, laboratory techniques; Northern blot--electrophoretic techniques, genetic techniques, laboratory techniques ; gene cloning--laboratory techniques, genetic techniques; yeast two-hybrid method--laboratory techniques, genetic techniques; pull-down assay--laboratory techniques, genetic techniques
MISCELLANEOUS TERMS: phenotype; type III secretion system
CONCEPT CODES:
03502 Genetics - General
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10064 Biochemistry studies - Proteins, peptides and amino acids
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae
06508 Pseudomonadaceae

A chaperone **-like HrpG protein acts as a suppressor of HrpV in regulation of the Pseudomonas syringae...**

ABSTRACT: The cloned **hrp** /hrc cluster of Pseudomonas syringae pv. syringae 61 (Pss61) contains 28 proteins, and many of...

...cytoplasmic protein whose predicted structure is similar to SicP

(E-value: 0.19), a TTSS **chaperone** of Salmonella typhimurium. Two non-polar hrpG mutants, Pss61-N826 and Pss61-N674, were produced...

...hrpV that codes for a negative regulator. Interestingly, a plasmid-borne hrpG can derepress the **hrp** expression in Pss61-N674 and in Pss61 overexpressing HrpV without decreasing the hrpV transcript. Moreover...

...suppressor of the negative regulator HrpV mediated via protein-protein interaction, leading to modulation of **hrp** /hrc expression subsequently freeing HrpS to promote the activation of other downstream **hrp** /hrc genes.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **chaperone** -like
GENE NAME: Pseudomonas syringae **hrp** gene cluster (Pseudomonadaceae...

4/9,K/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18366278 BIOSIS NO.: 200510060778
The Hrp **pathogenicity island of Erwinia amylovora and identification of three novel genes required for systemic infection**
AUTHOR: Oh Chang-Sik; Kim Jihyun F; Beer Steven V (Reprint)
AUTHOR ADDRESS: Cornell Univ, Dept Plant Pathol, Ithaca, NY 14853 USA**USA
AUTHOR E-MAIL ADDRESS: svb1@cornell.edu
JOURNAL: Molecular Plant Pathology 6 (2): p125-138 MAR 05 2005
ISSN: 1464-6722
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Sequence analysis of the region bordering the hrpldsp gene cluster of Erwinia amylovora strain Ea321, which causes fire blight, revealed characteristics of pathogenicity islands (PAIs). included are genes for a phage integrase, a tRNA(Phe), several orthologues of genes of YAPI, a PAI of Yersinia pseudotuberculosis, and several putative virulence genes with HrpL-dependent promoter motifs. The island is designated the **Hrp** PAI of E. amylovora. It is comprised of a chromosomal region of c. 62 kb with 60 open reading frames (ORF's). Comparison of the **Hrp** PAI of E amylovora with those of four closely related bacteria showed that orfB, a homologue of avrBsT of Xanthomonas campestris pv. vesicatoria, and orfA, its putative **chaperone** gene, are present only in the **Hrp** PAI of E. amylovora. As regions flanking the hrpldsp gene cluster are quite diverse, addition and deletion may have occurred during divergent evolution of the five bacteria. Among ORFs of the PAI of Ea321, three new HrpL-dependent genes were identified. Because they are required for full virulence in apple, they were designated hsvC, hsvB and hsvA (**hrp** -associated systemic virulence). They encode a homologue of an amidinotransferase for phaseolotoxin biosynthesis and homologues of a nikkomycin-biosynthetic protein of Pseudomonas syringae.

REGISTRY NUMBERS: 52350-85-3: integrase; 9031-64-5: amidinotransferase; 86003-55-6: nikkomycin; 62249-77-8: phaseolotoxin
DESCRIPTORS:
MAJOR CONCEPTS: Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic

Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Yersinia pseudotuberculosis (Enterobacteriaceae)--pathogen; Erwinia amylovora (Enterobacteriaceae)--pathogen, strain-Ea321; Pseudomonas syringae (Pseudomonadaceae)--pathogen; Xanthomonas campestris (Pseudomonadaceae)--pathovar-vesicatoria
ORGANISMS: PARTS ETC: chromosome
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganism
DISEASES: fire blight--bacterial disease, genetics
CHEMICALS & BIOCHEMICALS: integrase; open reading frames {ORFs}; transfer RNA {tRNA}; amidinotransferase; nikkomycin; **Hrp** pathogenicity island; phaseolotoxin
GENE NAME: Erwinia amylovora **hrp** /dsp gene cluster (Enterobacteriaceae); Erwinia amylovora orfA gene (Enterobacteriaceae); Erwinia amylovora orfB gene (Enterobacteriaceae); Erwinia amylovora hsvC gene (Enterobacteriaceae); Erwinia amylovora hsvB gene (Enterobacteriaceae); Erwinia amylovora hsvA gene (Enterobacteriaceae)
METHODS & EQUIPMENT: sequence analysis--laboratory techniques, genetic techniques
MISCELLANEOUS TERMS: divergent evolution; systemic infection
CONCEPT CODES:
03502 Genetics - General
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae
06508 Pseudomonadaceae

The Hrp **pathogenicity island of Erwinia amylovora and identification of three novel genes required for systemic infection**

...ABSTRACT: and several putative virulence genes with HrpL-dependent promoter motifs. The island is designated the **Hrp** PAI of E. amylovora. It is comprised of a chromosomal region of c. 62 kb with 60 open reading frames (ORF's). Comparison of the **Hrp** PAI of E amylovora with those of four closely related bacteria showed that orfB, a homologue of avrBsT of Xanthomonas campestris pv. vesicatoria, and orfA, its putative **chaperone** gene, are present only in the **Hrp** PAI of E. amylovora. As regions flanking the hrpldsp gene cluster are quite diverse, addition...

...they are required for full virulence in apple, they were designated hsvC, hsvB and hsvA (**hrp** -associated systemic virulence). They encode a homologue of an amidinotransferase for phaseolotoxin biosynthesis and homologues...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **Hrp** pathogenicity island
GENE NAME: Erwinia amylovora **hrp** /dsp gene cluster (Enterobacteriaceae ...

4/9,K/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18252305 BIOSIS NO.: 200500158477

Type III secretion chaperones ShcS1 and ShcO1 from Pseudomonas syringae pv. tomato DC3000 bind more than one effector

AUTHOR: Kabisch Ute; Landgraf Angelika; Krause Jana; Bonas Ulla; Boch Jens (Reprint)
AUTHOR ADDRESS: Genet Inst, Univ Halle Wittenberg, D-06099, Halle, Saale, Germany**Germany
AUTHOR E-MAIL ADDRESS: boch@genetik.uni-halle.de
JOURNAL: Microbiology (Reading) 151 (Part 1): p269-280 January 2005 2005
MEDIUM: print
ISSN: 1350-0872 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The **hrp** -type III secretion (TTS) system is a key pathogenicity factor of the plant pathogen Pseudomonas syringae pv. tomato DC3000 that translocates effector proteins into the cytosol of the eukaryotic host cell. The translocation of a subset of effectors is dependent on specific chaperones. In this study an operon encoding a TTS **chaperone** (ShcS1) and the truncated effector HopS1' was characterized. Yeast two-hybrid analysis and pull-down assays demonstrated that these proteins interact. Using protein fusions to AvrRpt2 it was shown that ShcS1 facilitates the translocation of HopS1', suggesting that ShcS1 is a TTS **chaperone** for HopS1' and that amino acids 1 to 118 of HopS1' are required for translocation. P. syringae pv. tomato DC3000 carries two shcS1 homologues, shcO1 and shcS2, which are located in different operons, and both operons include additional putative effector genes. Transcomplementation experiments showed that ShcS1 and ShcO1, but not ShcS2, can facilitate the translocation of HopS1' :: AvrRpt2. To characterize the specificities of the putative chaperones, yeast two-hybrid interaction studies were performed between the three chaperones and putative target effectors. These experiments showed that both ShcS1 and ShcO1 bind to two different effectors, HopS1' and HopO1-1, that share only 16% amino acid sequence identity. Using gel filtration it was shown that ShcS1 forms homodimers, and this was confirmed by yeast two-hybrid experiments. In addition, ShcS1 is also able to form heterodimers with ShcO1. These data demonstrate that ShcS1 and ShcO1 are exceptional class A TTS chaperones because they can bind more than one target effector.

DESCRIPTORS:
MAJOR CONCEPTS: Genetics; Infection; Methods and Techniques
BIOSYSTEMATIC NAMES: Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Pseudomonas syringae (Pseudomonadaceae)--pathogen, pathovar-tomato, strain-DC3000
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: Shc01 **chaperone** --binding; ShcS1 **chaperone** --binding; TTS **chaperone** ; amino acids; effector protein; homodimers; **hrp** type III secretion system
GENE NAME: Pseudomonas syringae Shc01 gene (Pseudomonadaceae)--binding, **chaperone** gene, expression c; Pseudomonas syringae ShcS1 gene (Pseudomonadaceae)--binding, **chaperone** gene, expression
METHODS & EQUIPMENT: gel filtration--chromatographic techniques, laboratory techniques; transcomplementation experiment--genetic techniques, laboratory techniques
CONCEPT CODES:

03502 Genetics - General
10064 Biochemistry studies - Proteins, peptides and amino acids
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
06508 Pseudomonadaceae
ABSTRACT: The **hrp** -type III secretion (TTS) system is a key pathogenicity factor of the plant pathogen Pseudomonas...

...of effectors is dependent on specific chaperones. In this study an operon encoding a TTS **chaperone** (ShcS1) and the truncated effector HopS1' was characterized. Yeast two-hybrid analysis and pull-down...

...was shown that ShcS1 facilitates the translocation of HopS1', suggesting that ShcS1 is a TTS **chaperone** for HopS1' and that amino acids 1 to 118 of HopS1' are required for translocation...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: Shc01 **chaperone** --...

...ShcS1 **chaperone** --...

...TTS **chaperone** ; ...

... **hrp** type III secretion system
...GENE NAME: binding, **chaperone** gene, expression c...

...binding, **chaperone** gene, expression

4/9,K/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18214579 BIOSIS NO.: 200500121644
Type III secretion chaperones of Pseudomonas syringae protect effectors from Lon-associated degradation
AUTHOR: Losada Liliana C; Hutcheson Steven W (Reprint)
AUTHOR ADDRESS: Dept Mol Genet and Cell Biol, Univ Maryland, College Pk, MD, 20742, USA**USA
AUTHOR E-MAIL ADDRESS: hutcheso@umd.edu
JOURNAL: Molecular Microbiology 55 (3): p941-953 February 2005 2005
MEDIUM: print
ISSN: 0950-382X _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The **hrp** type III secretion system (TTSS) of Pseudomonas syringae translocates effector proteins into the cytoplasm of host cells. Proteolysis of HrpR by Lon has been shown to negatively regulate the **hrp** TTSS. The inability to bypass Lon-associated effects on the regulatory system by ectopic expression of the known regulators suggested a second site of action for Lon in TTSS-dependent effector secretion. In this study we report that TTSS-dependent effectors are subject to the proteolytic degradation that appears to be rate-limiting to secretion. The half-lives of the effectors AvrPto, AvrRpt2, HopPsyA, HopPsyB1, HopPtoB2, HopPsyV1, HopPtoG and HopPtoM were substantially higher in

bacteria lacking Lon. TTSS-dependent secretion of several effectors was enhanced from Lon mutants. A primary role for chaperones appears to be protection of effectors from Lon-associated degradation prior to secretion. When coexpressed with their cognate **chaperone** , HopPsyB1, HopPsyV1 and HopPtoM were at least 10 times more stable in strains expressing Lon. Distinct Lon-targeting and **chaperone** -binding domains were identified in HopPtoM. The results imply that Lon is involved at two distinct levels in the regulation of the P. syringae TTSS: regulation of assembly of the secreton and modulation of effector secretion.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology
BIOSYSTEMATIC NAMES: Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Pseudomonas syringae (Pseudomonadaceae)--pathogen
ORGANISMS: PARTS ETC: cytoplasm
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: AvrPto; AvrRpt2; HopPsyA; HopPsyB1--expression; HopPsyV1--expression; HopPtoB2; HopPtoG; HopPtoM--expression; HrpR protein--proteolysis; Lon; **chaperone** ; effector protein--translocation; type III secretion system
CONCEPT CODES:
02502 Cytology - General
10060 Biochemistry studies - General
30500 Morphology and cytology of bacteria
31000 Physiology and biochemistry of bacteria
BIOSYSTEMATIC CODES:
06508 Pseudomonadaceae

ABSTRACT: The **hrp** type III secretion system (TTSS) of Pseudomonas syringae translocates effector proteins into the cytoplasm of host cells. Proteolysis of HrpR by Lon has been shown to negatively regulate the **hrp** TTSS. The inability to bypass Lon-associated effects on the regulatory system by ectopic expression...

...protection of effectors from Lon-associated degradation prior to secretion. When coexpressed with their cognate **chaperone** , HopPsyB1, HopPsyV1 and HopPtoM were at least 10 times more stable in strains expressing Lon. Distinct Lon-targeting and **chaperone** -binding domains were identified in HopPtoM. The results imply that Lon is involved at two ...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **chaperone** ;

4/9,K/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

18126788 BIOSIS NO.: 200500033853
HpaB from Xanthomonas campestris pv. vesicatoria acts as an exit control protein in type III-dependent protein secretion
AUTHOR: Buettner Daniela (Reprint); Guerlebeck Doreen; Noel Laurent D; Bonas Ulla
AUTHOR ADDRESS: Inst Genet, Univ Halle Wittenberg, D-06099, Halle, Saale, Germany**Germany
AUTHOR E-MAIL ADDRESS: buettner@genetik.uni-halle.de

JOURNAL: Molecular Microbiology 54 (3): p755-768 November 2004 2004
MEDIUM: print
ISSN: 0950-382X _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The **hrp** (hypersensitive response and pathogenicity) gene cluster of the plant pathogenic bacterium Xanthomonas campestris pv. vesicatoria encodes a type III secretion (TTS) system, which injects bacterial effector proteins into the plant cell. Here, we characterized hpaB (hpa, **hrp** -associated), which encodes a pathogenicity factor with typical features of a TTS **chaperone** . We show that HpaB is important for the efficient secretion of at least five effector proteins but is dispensable for the secretion of non-effectors such as XopA and the TTS translocon protein HrpF. GST pull-down assays revealed that HpaB interacts with two unrelated effector proteins, AvrBs1 and AvrBs3, but not with XopA. The HpaB-binding site is located within the first 50 amino acids of AvrBs3. This region also contains the targeting signal for HpaB-dependent secretion, which is missing in HrpF and XopA. Intriguingly, the N-termini of HrpF and XopA target the AvrBs3DELTA2 reporter for translocation in a DELTAhpaB mutant but not in the wild-type strain. This indicates that HpaB plays an essential role in the exit control of the TTS system. Our data suggest that HpaB promotes the secretion of a large set of effector proteins and prevents the delivery of non-effectors into the plant cell.

REGISTRY NUMBERS: 50812-37-8: GST
ENZYME COMMISSION NUMBER: EC 2.5.1.18: GST
DESCRIPTORS:
MAJOR CONCEPTS: Genetics; Infection; Molecular Genetics--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Xanthomonas campestris (Pseudomonadaceae)--pathogen, phagovar-vesicatoria, strain-75-3
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: AvrBs1; AvrBs3; GST {glutathione S-transferase}; HpaB--exit control protein; HrpF protein; RNA; XopA; cDNA {complementary DNA}; type III secretion system {TTS}
MOLECULAR SEQUENCE DATABANK NUMBER: AAD21328--NCBI, amino acid sequence, nucleotide sequence; AAM35287--NCBI, amino acid sequence, nucleotide sequence; AAM40518--NCBI, amino acid sequence, nucleotide sequence; AAP34355--NCBI, amino acid sequence, nucleotide sequence; AAS48655--NCBI, amino acid sequence, nucleotide sequence; AAT44860--NCBI, amino acid sequence, nucleotide sequence; AF056246--NCBI, amino acid sequence, nucleotide sequence; CAD18004--NCBI, amino acid sequence, nucleotide sequence
GENE NAME: Xanthomonas campestris hpaB gene (Pseudomonadaceae); Xanthomonas campestris **hrp** gene cluster (Pseudomonadaceae) { Xanthomonas campestris hypersensitive response and pathogenicity gene cluster}; Xanthomonas campestris hrpE gene (Pseudomonadaceae)
CONCEPT CODES:
03502 Genetics - General
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10064 Biochemistry studies - Proteins, peptides and amino acids
10802 Enzymes - General and comparative studies: coenzymes

31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
06508 Pseudomonadaceae

ABSTRACT: The **hrp** (hypersensitive response and pathogenicity) gene cluster of the plant pathogenic bacterium Xanthomonas campestris pv. vesicatoria...

...system, which injects bacterial effector proteins into the plant cell. Here, we characterized hpaB (hpa, **hrp** -associated), which encodes a pathogenicity factor with typical features of a TTS **chaperone** . We show that HpaB is important for the efficient secretion of at least five effector...
DESCRIPTORS:
...GENE NAME: Xanthomonas campestris **hrp** gene cluster (Pseudomonadaceae) {Xanthomonas campestris hypersensitive response and pathogenicity gene cluster...

4/9,K/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17905464 BIOSIS NO.: 200400276221
The HopPtoF locus of Pseudomonas syringae pv. tomato DC3000 encodes a type III chaperone **and a cognate effector**
AUTHOR: Shan Libo; Oh Hye-Sook; Chen Jianfu; Guo Ming; Zhou Jianmin; Alfano James R; Collmer Alan; Jia Xu; Tang Xiaoyan (Reprint)
AUTHOR ADDRESS: Dept Plant Pathol, Kansas State Univ, Throckmorton Hall, Manhattan, KS, 66506, USA**USA
AUTHOR E-MAIL ADDRESS: xtang@ksu.edu
JOURNAL: Molecular Plant-Microbe Interactions 17 (5): p447-455 May 2004 2004
MEDIUM: print
ISSN: 0894-0282 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Type III secretion systems are highly conserved among gram-negative plant and animal pathogenic bacteria. Through the type III secretion system, bacteria inject a number of virulence proteins into the host cells. Analysis of the whole genome sequence of Pseudomonas syringae pv. tomato DC3000 strain identified a locus, named HopPtoF, that is homologous to the avirulence gene locus avrPphF in R syringae pv. phaseolicola. The HopPtoF locus harbors two genes, ShcFPto and HopFPto that are preceded by a single **hrp** box promoter. We present evidence here to show that ShcFPto and HopFPto encode a type III **chaperone** and a cognate effector, respectively. ShcFPto interacts with and stabilizes the HopFPto protein in the bacterial cell. Translation of HopFPto starts at a rare initiation codon ATA that limits the synthesis of the HopFPto protein to a low level in bacterial cells.

DESCRIPTORS:
MAJOR CONCEPTS: Infection; Molecular Genetics--Biochemistry and Molecular Biophysics

BIOSYSTEMATIC NAMES: Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms
ORGANISMS: Pseudomonas syringae (Pseudomonadaceae)--pathogen, pathovar-tomato, strain-DC3000
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms
CHEMICALS & BIOCHEMICALS: ATA initiation codon; HopPtoF locus; cognate effector; type III **chaperone**
CONCEPT CODES:
03502 Genetics - General
31000 Physiology and biochemistry of bacteria
31500 Genetics of bacteria and viruses
BIOSYSTEMATIC CODES:
06508 Pseudomonadaceae

The HopPtoF locus of Pseudomonas syringae pv. tomato DC3000 encodes a type III chaperone **and a cognate effector**

...ABSTRACT: The HopPtoF locus harbors two genes, ShcFPto and HopFPto that are preceded by a single **hrp** box promoter. We present evidence here to show that ShcFPto and HopFPto encode a type III **chaperone** and a cognate effector, respectively. ShcFPto interacts with and stabilizes the HopFPto protein in the...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...type III **chaperone**

4/9,K/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17517155 BIOSIS NO.: 200300471110
Pseudomonas syringae pv. tomato DC3000 HopPtoM (CEL ORF3) is important for lesion formation but not growth in tomato and is secreted and translocated by the Hrp **type III secretion system in a** chaperone **-dependent manner.**
AUTHOR: Badel Jorge L; Nomura Kinya; Bandyopadhyay Sruti; Shimizu Rena; Collmer Alan; He Sheng Yang (Reprint)
AUTHOR ADDRESS: Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI, 48824, USA**USA
AUTHOR E-MAIL ADDRESS: hes@msu.edu
JOURNAL: Molecular Microbiology 49 (5): p1239-1251 September 2003 2003
MEDIUM: print
ISSN: 0950-382X _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Pseudomonas syringae pv. tomato DC3000 is a pathogen of tomato and Arabidopsis that injects virulence effector proteins into host cells via a type III secretion system (TTSS). TTSS-deficient mutants have a **Hrp** -phenotype, that is, they cannot elicit the hypersensitive response (HR) in non-host plants or pathogenesis in host plants. Mutations in effector genes typically have weak virulence phenotypes (apparently due to redundancy), but deletion of six open reading frames (ORF) in the DC3000 conserved effector locus (CEL) reduces parasitic growth and abolishes disease symptoms without affecting function of the TTSS. The inability of the DELTACEL mutant to cause disease symptoms in tomato was

restored by a clone expressing two of the six ORF that had been deleted: CEL ORF3 (HopPtoM) and ORF4 (ShcM). A DELTAhopPtoM::nptII mutant was constructed and found to grow like the wild type in tomato but to be strongly reduced in its production of necrotic lesion symptoms. HopPtoM expression in DC3000 was activated by the HrpL alternative sigma factor, and the protein was secreted by the **Hrp** TTSS in culture and translocated into Arabidopsis cells by the **Hrp** TTSS during infection. Secretion and translocation were dependent on ShcM, which was neither secreted nor translocated but, like typical TTSS chaperones, could be shown to interact with HopPtoM, its cognate effector, in yeast two-hybrid experiments. Thus, HopPtoM is a type III effector that, among known plant pathogen effectors, is unusual in making a major contribution to the elicitation of lesion symptoms but not growth in host tomato leaves.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Infection
BIOSYSTEMATIC NAMES: Pseudomonadaceae--Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms; Solanaceae--Dicotyledones, Angiospermae, Spermatophyta, Plantae
ORGANISMS: Pseudomonas syringae (Pseudomonadaceae)--pathovar-tomato DC3000; tomato (Solanaceae)--host
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Angiosperms ; Dicots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: HopPtoM; **Hrp** type III secretion system
METHODS & EQUIPMENT: yeast two-hybrid experiments--genetic techniques, laboratory techniques
MISCELLANEOUS TERMS: lesion formation; parasitic growth
CONCEPT CODES:
10060 Biochemistry studies - General
31000 Physiology and biochemistry of bacteria
51522 Plant physiology - Chemical constituents
BIOSYSTEMATIC CODES:
06508 Pseudomonadaceae
26775 Solanaceae

...**for lesion formation but not growth in tomato and is secreted and translocated by the** Hrp **type III secretion system in a** chaperone **-dependent manner.**

...ABSTRACT: into host cells via a type III secretion system (TTSS). TTSS-deficient mutants have a **Hrp** -phenotype, that is, they cannot elicit the hypersensitive response (HR) in non-host plants or...

...was activated by the HrpL alternative sigma factor, and the protein was secreted by the **Hrp** TTSS in culture and translocated into Arabidopsis cells by the **Hrp** TTSS during infection. Secretion and translocation were dependent on ShcM, which was neither secreted nor...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **Hrp** type III secretion system

4/9,K/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17403150 BIOSIS NO.: 200300361869
A novel chaperone **to stabilize Horseradish Peroxidase (** HRP **) based**

conjugates in ELISA products: An alternate plant protein to Bovine Serum Albumin (BSA).
AUTHOR: Mehra M (Reprint); DeAnda A (Reprint); Draviam E (Reprint); Ponni Y ; Wolf A; Wang W; Shosegov O; Altman A
AUTHOR ADDRESS: Biotecx, Houston, TX, USA**USA
JOURNAL: Clinical Chemistry 49 (S6): pA117 June 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 55th Annual Meeting of the AACC (American Association for Clinical Chemistry) Philadelphia, PA, USA July 20-24, 2003; 20030720
SPONSOR: American Association for Clinical Chemistry
ISSN: 0009-9147
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 67-64-1: acetone; 50-01-1: guanidine hydrochloride; 9003-99-0: horseradish peroxidase; 39450-01-6: proteinase K; 9002-07-7: trypsin
ENZYME COMMISSION NUMBER: EC 1.11.1.7: horseradish peroxidase; EC 3.4.21.64 : proteinase K; EC 3.4.21.4: trypsin
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Salicaceae--Dicotyledones, Angiospermae, Spermatophyta, Plantae
ORGANISMS: Escherichia coli (Enterobacteriaceae)--expression system; Populus tremula {aspen tree} (Salicaceae)
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Angiosperms ; Dicots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: SDS; SP1-- **chaperone** -like protein; acetone; bovine serum albumin {BSA}; guanidine hydrochloride; horseradish peroxidase--conjugates; phosphate-buffered saline; protein; proteinase K; trypsin
METHODS & EQUIPMENT: Biotecx OptiCoat enzyme immunoassay kit {Biotecx OptiCoat EIA kit}--laboratory kit; ELISA--immunologic techniques, laboratory techniques; Toxoplasma IgG ELISA kit {Toxoplasma immunoglobulin G ELISA kit}--laboratory kit; enzyme immunoassay {EIA}--immunologic techniques, laboratory techniques
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10802 Enzymes - General and comparative studies: coenzymes
31000 Physiology and biochemistry of bacteria
51522 Plant physiology - Chemical constituents
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae
26695 Salicaceae

A novel chaperone **to stabilize Horseradish Peroxidase (HRP) based conjugates in ELISA products: An alternate plant protein to Bovine Serum Albumin (BSA).**
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **chaperone** -like protein
? s horseradish(w)peroxidase
105851 HORSERADISH
379989 PEROXIDASE
S5 102299 HORSERADISH(W)PEROXIDASE

```
? s s1 and s5
           59277  S1
          102299  S5
      S6      70  S1 AND S5
? s s1(s)s5
           59277  S1
          102299  S5
      S7      54  S1(S)S5
? s s6 and protect?
              70  S6
         2724613  PROTECT?
      S8       4  S6 AND PROTECT?
? s s7 and protect?
              54  S7
         2724613  PROTECT?
      S9       3  S7 AND PROTECT?
? t s8/9,k/1-4
```

8/9,K/1 (Item 1 from file: 5)

17184730 BIOSIS NO.: 200300143449

Distribution of Heme Oxygenase-1 (HO-1) in Rabbit Cornea.

AUTHOR: Chang R I (Reprint); McCanna D; Kalsow C M
AUTHOR ADDRESS: Ophthalmology, University Rochester, Rochester, NY, USA** USA

JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002 p Abstract No. 1678 2002 2002
MEDIUM: cd-rom
CONFERENCE/MEETING: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002; 20020505
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Purpose: Heme oxygenase-1 (HO-1) also named heat shock protein 32 (Hsp32) is a molecular **chaperone** protein that can be induced in response to increased oxidative stress, hypoxia, heme, metals or inflammatory cytokines. Upregulation of HO-1 mRNA and protein have been measured in rabbit corneal epithelia in response to heme, metals, and inflammatory cytokines as well as hypoxia and oxidative stress (Biochem. Pharmacol. 53:1069, 1997). Homogenization of tissue for these quantitative studies precludes any localization of HO-1. The current immunohistochemical study demonstrates the distribution of HO-1 in rabbit cornea. Method: Corneas of 10 New Zealand white rabbits (2.5-3.5kg) were fixed in 95% ethanol and embedded in paraffin. HO-1 was detected using mouse anti-heme-oxygenase-1 monoclonal antibody (MAb HO-1) as the primary antibody. Distribution of immunoreactivity was visualized with a **horseradish peroxidase** mouse Envision+ System with DAB substrate. Specificity of reactivity was confirmed by comparison of reactivity patterns for other heat shock proteins, Hsp 47 and inducible and constitutive Hsp70. Results: HO-1 immunoreactivity in the corneal epithelium was primarily observed in the cytoplasm of basal epithelial cells. The reactivity throughout the corneal epithelium was not uniform, i.e., some areas had no reactivity while other areas had reactivity

throughout the epithelium including wing and superficial cells. MAb HO-1 reacted consistently and uniformly with limbal vascular endothelium. The epithelial and endothelial patterns of reactivity with MAb HO-1 were distinct from those of the other hsp's tested. Conclusion: These experiments qualitatively demonstrate the distribution of HO-1 in normal rabbit cornea. The variability of expression in different areas of the corneal epithelium indicates the responsive nature of HO-1 expression to changes in the oxidative state of the microenvironment. Communication between the different layers of epithelium and the change in molecular machinery as cells mature from the basal layer to the more superficial area could play a role in the rapid response. Since corneal epithelium and limbal vascular endothelium are at the interface of the cornea with the external environment and the internal (vascular) environment of the cornea, HO-1 is a potentially important **protective** mechanism for this tissue. HO-1 expression may be exploited as an early indicator of corneal oxidative stress and/or as a means to enhance **protection** from oxidative stress. Support: Rochester Eye and Human Parts Bank and Bausch and Lomb, Inc.

REGISTRY NUMBERS: 9003-99-0: **horseradish peroxidase**
ENZYME COMMISSION NUMBER: EC 1.11.1.7: **horseradish peroxidase**
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology--Biochemistry and Molecular Biophysics; Sense Organs--Sensory Reception
BIOSYSTEMATIC NAMES: Leporidae--Lagomorpha, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: rabbit (Leporidae); New Zealand white rabbit (Leporidae)
ORGANISMS: PARTS ETC: cornea--sensory system; corneal epithelium--sensory system
COMMON TAXONOMIC TERMS: Animals; Chordates; Lagomorphs; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Vertebrates
CHEMICALS & BIOCHEMICALS: heme oxygenase-1 {HO-1}; heat shock protein 32 {Hsp32}; cytokines; anti-heme-oxygenase-1 monoclonal antibody; **horseradish peroxidase**
METHODS & EQUIPMENT: immunohistochemical study--immunologic techniques, laboratory techniques
MISCELLANEOUS TERMS: oxidative stress; Meeting Abstract
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10802 Enzymes - General and comparative studies: coenzymes
20004 Sense organs - Physiology and biochemistry
BIOSYSTEMATIC CODES:
86040 Leporidae

...ABSTRACT: Heme oxygenase-1 (HO-1) also named heat shock protein 32 (Hsp32) is a molecular **chaperone** protein that can be induced in response to increased oxidative stress, hypoxia, heme, metals or...

...antibody (MAb HO-1) as the primary antibody. Distribution of immunoreactivity was visualized with a **horseradish peroxidase** mouse Envision+ System with DAB substrate. Specificity of reactivity was confirmed by comparison of reactivity...
...environment and the internal (vascular) environment of the cornea, HO-1 is a potentially important **protective** mechanism for this tissue. HO-1 expression may be exploited as an early indicator of corneal oxidative stress and/or as a means to enhance **protection** from oxidative stress. Support: Rochester Eye and Human Parts Bank and Bausch and Lomb, Inc.

...REGISTRY NUMBERS: **horseradish peroxidase**
...ENZYME COMMISSION NUMBER: **horseradish peroxidase**
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **horseradish peroxidase**

8/9,K/2 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

20240283 PMID: 15745946
The multiple functions of cysteine-string protein analyzed at Drosophila nerve terminals.
Bronk Peter; Nie Zhiping; Klose Markus K; Dawson-Scully Ken; Zhang Jinhui ; Robertson R Meldrum; Atwood Harold L; Zinsmaier Konrad E
Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, Arizona 85721-0077, USA.
Journal of neuroscience - the official journal of the Society for Neuroscience (United States) Mar 2 2005, 25 (9) p2204-14, ISSN 1529-2401--Electronic Journal Code: 8102140
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The synaptic vesicle-associated cysteine-string protein (CSP) is important for synaptic transmission. Previous studies revealed multiple defects at neuromuscular junctions (NMJs) of csp null-mutant Drosophila, but whether these defects are independent of each other or mechanistically linked through J domain mediated-interactions with heat-shock cognate protein 70 (Hsc70) has not been established. To resolve this issue, we genetically dissected the individual functions of CSP by an in vivo structure/function analysis. Expression of mutant CSP lacking the J domain at csp null-mutant NMJs fully restored normal thermo-tolerance of evoked transmitter release but did not completely restore evoked release at room temperature and failed to reverse the abnormal intraterminal Ca2+ levels. This suggests that J domain-mediated functions are essential for the regulation of intraterminal Ca2+ levels but only partially required for regulating evoked release and not required for **protecting** evoked release against thermal stress. Hence, CSP can also act as an Hsc70-independent **chaperone protecting** evoked release from thermal stress. Expression of mutant CSP lacking the L domain restored neurotransmission and partially reversed the abnormal intraterminal Ca2+ levels, suggesting that the L domain is important, although not essential, for the role of CSP in regulating intraterminal Ca2+ levels. We detected no effects of csp mutations on individual presynaptic Ca2+ signals triggered by action potentials, suggesting that presynaptic Ca2+ entry is not primarily impaired. Both the J and L domains were also required for the role of CSP in synaptic growth. Together, these results suggest that CSP has several independent synaptic functions, affecting synaptic growth, evoked release, thermal **protection** of evoked release, and intraterminal Ca2+ levels at rest and during stimulation.
Descriptors: *HSP40 Heat-Shock Proteins--physiology--PH; *Membrane Proteins--physiology--PH; *Neuromuscular Junction--cytology--CY; *Point Mutation--physiology--PH; *Presynaptic Terminals--metabolism--ME; Analysis

of Variance; Animals; Animals, Genetically Modified; Calcium--metabolism --ME; Calcium Signaling--physiology--PH; Comparative Study; Diagnostic Imaging--methods--MT; Drosophila; Drosophila Proteins--metabolism--ME; Evolution; Gene Expression--genetics--GE; HSP40 Heat-Shock Proteins --chemistry--CH; HSP40 Heat-Shock Proteins--genetics--GE; **Horseradish Peroxidase** --metabolism--ME; Humans; Immunohistochemistry--methods--MT; Membrane Potentials--physiology--PH; Membrane Proteins--chemistry--CH; Membrane Proteins--genetics--GE; Neuromuscular Junction--metabolism--ME; Neuromuscular Junction--physiology--PH; Patch-Clamp Techniques--methods --MT; Presynaptic Terminals--physiology--PH; Protein Structure, Tertiary --genetics--GE; Protein Structure, Tertiary--physiology--PH; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.; Structure-Activity Relationship; Temperature; Time Factors

CAS Registry No.: 0 (Drosophila Proteins); 0 (HSP40 Heat-Shock Proteins); 0 (Membrane Proteins); 0 (cysteine string protein); 7440-70-2 (Calcium)

Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)

Record Date Created: 20050304

Record Date Completed: 20060331

... intraterminal Ca2+ levels but only partially required for regulating evoked release and not required for **protecting** evoked release against thermal stress. Hence, CSP can also act as an Hsc70-independent **chaperone protecting** evoked release from thermal stress. Expression of mutant CSP lacking the L domain restored neurotransmission...

... results suggest that CSP has several independent synaptic functions, affecting synaptic growth, evoked release, thermal **protection** of evoked release, and intraterminal Ca2+ levels at rest and during stimulation.

...; Expression--genetics--GE; HSP40 Heat-Shock Proteins--chemistry--CH; HSP40 Heat-Shock Proteins--genetics--GE; **Horseradish Peroxidase** --metabolism--ME; Humans; Immunohistochemistry--methods--MT; Membrane Potentials--physiology--PH; Membrane Proteins--chemistry--CH; Membrane...

Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)

Chemical Name: Drosophila Proteins; HSP40 Heat-Shock Proteins; Membrane Proteins; cysteine string protein; Calcium; **Horseradish Peroxidase**

8/9,K/3 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0338849 DBR Accession No.: 2004-11141 PATENT

Novel isolated denaturant which is e.g. boiling- or detergent-stable and/or protease resistant protein having chaperone **-like activity, useful for strengthening hair, nail or skin and for inducing wound healing - recombinant enzyme protein production for use in disease therapy and plant engineering**

AUTHOR: WANG W; PELAH D; ALEGRAND T; SHOSEYOV O; ALTMAN A; POUNY Y; MARTON I; WOLF A

PATENT ASSIGNEE: YISSUM RES DEV CO HEBREW UNIV JERUSALEM; FULCRUM SP LTD 2004

PATENT NUMBER: WO 200422697 PATENT DATE: 20040318 WPI ACCESSION NO.: 2004-248452 (200423)

PRIORITY APPLIC. NO.: US 233409 APPLIC. DATE: 20020904

NATIONAL APPLIC. NO.: WO 2003IL723 APPLIC. DATE: 20030902

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) having **chaperone** -like activity and **horseradish peroxidase** (HRP) **protection** activity, as determined by HRP **protection** assay, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid (II) comprising a first polynucleotide encoding (I), and a second polynucleotide including a promoter sequence operably linked to the first polynucleotide for directing an expression of (I); (2) a nucleic acid construct comprising (II); (3) a cell transformed with (II); (4) an organism transformed with (II); (5) isolating a gene encoding (I) from a biological source, involves screening an expression library with a polynucleotide encoding (I); (6) an antibody (III) recognizing one or more epitopes of (I); (7) enriching or isolating (M1) denaturant stable (boiling stable or detergent stable) and/or protease resistant protein from a biological source, involves extracting total proteins from the biological source to obtain a protein extract, boiling the protein extract, collecting the soluble proteins and optionally assaying for **chaperone** -like activity of the soluble proteins and enriching or isolating the stable protein having **chaperone** -like activity; (8) isolating a gene encoding (I) from a biological source; (9) identifying a nucleic acid potentially encoding (I), involves searching an electronic library containing several nucleic acid and/or amino acid sequences for sequences having a predetermined degree of identity or homology to any one of the nucleic acid sequences (N1) chosen from 5 fully defined sequences of 567, 593, 357, 497, 366 base pairs as given in the specification, or to any one of the amino acid sequences (A1) chosen from 26 fully defined sequences e.g., 108, 98, 98, 98, 84, 98, 98, 109, 47, 98, 98, 93 and 108 amino acids as given in the specification, or their portions or corresponding to at least 15 bases; (10) isolating a nucleic acid potentially encoding (I); (11) detergent-free isolation of a protease-resistant protein having **chaperone** -like activity from a biological source, involves extracting total proteins from the biological source, to obtain a protein extract, contacting the protein extract with a protease, and isolating a protease-resistant protein, and optionally assaying the protease-resistant protein for **chaperone** -like activity; (12) a fusion protein (IV) comprising (I) fused to an additional polypeptide; (13) a transgenic plant expressing (I) above a natural amount of (I) in the plant; (14) rendering a plant more tolerant to a biotic or abiotic stress, involves engineering the plant to express (I) above a natural amount of (I) in the plant; (15) rendering a plant more recoverable from a biotic or abiotic stress, involves engineering the plant to express (I) above a natural amount of (I) in the plant; (16) isolating (M2) a boiling stable protein from a biological source, involves carrying out extracting, and boiling steps of (M1), recovering soluble protein fraction, and optionally assaying the protease resistant protein for **chaperone** -like activity; (17) a pharmaceutical composition (V) comprising (I) as an active ingredient and a carrier; (18) a hetero complex (VI) comprising an oligomer including several of (I), and at least two different molecules being fused to the oligomer; and (19) increasing a specific activity of a pre-isolated (I) as determined in Units of **protecting** activity per mg protein, involves autoclaving the pre-isolated (I), or treating the pre-isolated (I) with a protease. BIOTECHNOLOGY - Preferred Protein: The isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) has **chaperone** -like activity and

horseradish peroxidase (HRP) **protection** activity, as determined by HRP **protection** assay, of at least 10 Units/mg protein, where the HRP **protection** assay comprises mixing (I) at different final protein concentrations at a predetermined volume with 100 microliters of 5 nM HRP present in 40 mM N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid (HEPES) buffer at pH 7.5, thus forming a first reaction mixture, and following incubation of the reaction mixture at 25 degreesC for 16

hours, determining HRP remaining enzymatic activity by mixing 5 microliters of the first reaction mixture with 100 microliters of 3,3',5,5'-tetramethylbenzidine, thus forming a second reaction mixture, incubating the second reaction mixture for 10 minutes, stopping a reaction of the second reaction mixture by an addition of 100 microliters of 1 M sulfuric acid and recording colorimetric change in the second reaction mixture at 435 nm, and units are defined as a dilution factor of (I) at a concentration of 1 mg/ml that confers 50% **protection** of HRP activity in the HRP **protection** assay. In (I), the HRP **protection** activity is of 15000, 10000, 8000, 6000, 5500, 5000, 4500, 4000, 3500, 3000, 2500, 2000, 1500, 1000 or at least 500 Units/mg protein. Preferred Nucleic Acid: In (II), the promoter sequence is a eukaryotic constitutive promoter. The promoter is a plant promoter chosen from a constitutive plant promoter, a tissue specific plant promoter and an inducible plant promoter. The constitutive plant promoter is chosen from Cauliflower mosaic virus (CaMV)35S plant promoter, CamV19S plant promoter, figwort mosaic virus (FMV)34S plant promoter, sugarcane bacilliform badnavirus plant promoter, CsVMV plant promoter, Arabidopsis ACT2/ACT8 actin plant promoter, Arabidopsis ubiquitin UBQ1 plant promoter, barley leaf thionin BTH6 plant promoter, and rice actin plant promoter. The tissue specific plant promoter is chosen from bean phaseolin storage protein plant promoter, DLEC plant promoter, PHSbeta plant promoter, zein storage protein plant promoter, conglutin gamma plant promoter from soybean, AT2S1 gene plant promoter, ACT11 actin plant promoter from Arabidopsis, napA plant promoter from Brassica napus and potato patatin gene plant promoter. The inducible plant promoter is chosen from a light-inducible plant promoter derived from the pea rbcS gene, a plant promoter from the alfalfa rbcS gene, DRE, MYC and MYB plant promoters, which are active in drought, INT, INPS, prxEa, Ha hsp17.7G4 and RD21 plant promoters active in high salinity and osmotic stress, and hsr203J and str246C plant promoters active in pathogenic stress. The first polynucleotide has a sequence at least 60% identical with 6 fully defined sequences of 567, 593, 357, 428, 497 or 366 base pairs as given in the specification, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9. (I) Has a sequence at least 60% identical to a fully defined sequence of 108 or 112 amino acids as given in the specification, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2. (I) Is natively an oligomer. The **chaperone** -like activity includes heat stabilization of proteins. (II) Further comprises a third polynucleotide encoding an additional protein, and being adjacent and in frame to the first polynucleotide, where the first and third polynucleotides encoding, in combination, a fusion protein of the (I) and the additional protein. Preferred Pharmaceutical Composition: (V) Is packaged in a package and identified in print for use in a wound

healing application or strengthening and/or grooming hair, nail or skin application. Preferred Method: Isolating a gene encoding (I) from a biological source, comprises: (a) carrying out the steps of extracting, boiling, collecting, assaying, and isolating of (M1), raising antibodies recognizing the stable protein having **chaperone** -like activity and screening an expression library with the antibodies; or (b) carrying out the steps of (M1), micro-sequencing the stable protein to obtain at least a partial amino acid sequence, designing an oligonucleotide corresponding to the amino acid sequence and screening a library with the oligonucleotide. Isolating a nucleic acid potentially encoding (I) comprises: (a) screening a cDNA or genomic library with a polynucleotide of at least 17 bases and at least 60% identical to a contiguous portion of 6 fully defined sequences of 567, 593, 357, 428, 497 or 366 base pairs as given in the specification; or (b) providing at least one pair of oligonucleotides each being at least 15 bases in length, including one or more oligonucleotides corresponding to (N1), and selected for amplifying a nucleic acid having a degree of identity with, or encoding proteins exhibiting homology to (A1), contacting at least one pair of oligonucleotides with a sample of nucleic acid and amplifying the nucleic acid having degree of identity with, or encoding proteins exhibiting homology to (A1), and using nucleic acid having degree of identity with, or encoding proteins exhibiting homology to (A1) for isolating a nucleic acid potentially encoding (I). (M2) further involves digesting the protein extract with a protease. Preferred Hetero Complex: In (VI), the two different molecules comprise a first enzyme and a second enzyme, that catalyze sequential or different reactions in a synthesis or degradation pathway. The two different molecules comprise at least a binding molecule and a reporter molecule. Preferred Fusion Protein: (I) Is fused to the additional polypeptide through a peptide bond or a cross-linker, where (IV) has an oligomeric form. ACTIVITY - Vulnerary; Nootropic; **Neuroprotective** . No biological data given. MECHANISM OF ACTION - Prevents aggregation of aggregating proteins; Inducer of immune response (claimed). USE - (I) Is useful for preventing an aggregating protein from aggregating into an aggregate, which involves causing an effective amount of (I) to become in contact with aggregating protein. (I) Is useful for de-aggregating aggregates of an aggregating protein, which involves causing an effective amount of (I) to become in contact with the aggregate. (I) Is useful for stabilizing a protein against denaturing conditions, which involves causing an effective amount of (I) to become in contact with the protein. (I) Is useful for **protecting** an enzyme preparation from reduction in enzymatic activity, which involves adding (I) to the enzyme preparation in an amount sufficient for **protecting** the enzyme preparation from reduction in enzymatic activity. (I) is useful for repairing at least a portion of lost enzymatic activity of an enzyme preparation, which involves adding (I) to the enzyme preparation, in an amount sufficient for repairing portion of the lost enzymatic activity of the enzyme preparation. (I) is useful for increasing cell migration, which involves exposing the cells to (I), in an amount sufficient for increasing cell migration. (I) is useful for accelerating or inducing wound healing, which involves administering (I) on to a wound, in an amount sufficient for accelerating or inducing wound healing. (I) is useful for strengthening or grooming hair, nail or skin, which involves administering (I) onto the hair, nail or skin sufficient for strengthening or grooming the hair, nail or skin. (I) is useful for treating a disease associated with protein aggregation of an

aggregating protein, which involves administering (I) to a subject who is in need, in an amount sufficient for de-aggregating and/or preventing aggregation of the aggregating protein such as beta-amyloid or prion. (I) is useful for increasing a binding avidity of a binding molecule, which involves displaying multiple copies of the binding molecule on a surface of an oligomer of (I), where the binding molecule is chosen from a receptor, ligand, enzyme, substrate, inhibitor, antibody or antigen. (I) is useful in administering a polypeptide to an animal having a immune system by reducing an immune response against the polypeptide, which involves administering the polypeptide being fused to (I), to the animal and thus reducing the immune response against the polypeptide as compared to the immune response that is developed by administering the polypeptide alone to the animal. (III) is useful for isolating gene encoding (I), which involves screening an expression library with (III). (IV) is useful in immunization, which involves subjecting an immune system of a mammal to (IV) (claimed). (I) is useful for treating a disease such as Alzheimer's disease and prion associated diseases e.g., encephalus spongyform, by preventing aggregation of aggregating proteins. ADVANTAGE - (I) retains its activity and oligomerability also when forming a fusion protein. EXAMPLE - Boiling stable protein fractions of aspen, tomato M82, VF36 and pine were prepared as follows: Crude plant extracts were centrifuged for 10 minutes and supernatants were transferred to fresh tubes. The supernatants were subjected to a 10-minutes boiling session, then kept on ice for 5 minutes and centrifuged for 10 minutes. Resulting supernatants were precipitated by adding 4 volumes of cold acetone, and centrifuged for 10 minutes. Boiling stable proteins were then recovered by dissolving the pellets in 10 mM Tris-hydrochloric acid buffer (pH 7.5). The total boiling-stable proteins were separated on a 17% sodium dodecyl sulfate (SDS)-tricine polyacrylamide gel electrophoresis (PAGE), during which two bands of 66 and 116 kDa band were obtained. The 66 kDa band was found to represent a germin-like protein. Acetone-precipitated boiling-stable proteins of aspen plant were dissolved in 1X tricine-SDS sample buffer (100 mM Tris-hydrochloric acid pH 6.8, 20% glycerol, 1% SDS, 0.025% Coomassie blue), and then separated on a preparative 17% polyacrylamide tricine-SDS gel. Major bands corresponding to stable proteins (SP)-1 (116 kDa oligomer and 12.4 kDa monomer) protein were excised from the gel. SP1 oligomer and monomer were electro-eluted separately, in a dialysis bag. The eluted product was further dialyzed against 500 volumes of 10 mM Tris-hydrochloric acid overnight at 4degreesC, followed by acetone precipitation and centrifugation. Purified SP1 was obtained by dissolving the pellet in 10 mM Tris-hydrochloric acid. The prepared stable protein when maintained with **horseradish peroxidase**, was found to maintain its activity. (176 pages)

DESCRIPTORS: recombinant denaturant stable protease resistant, **chaperone**-like protein, **horseradish peroxidase**, prep., vector-mediated gene transfer expression in host cell, antibody, fusion protein, appl. transgenic plant construction, biotic, abiotic stress tolerance, aggregating protein prevention, protein stabilization, enzyme loss act. repair, cell migration, wound healing induction, grooming hair, Alzheimer disease, prion associated disease, encephalus spongyform therapy plant Armoracia rusticana enzyme EC-1.11.1.7 crop improvement vulnerary nootropic **neuroprotective** DNA sequence protein sequence (23, 22)

SECTION: THERAPEUTICS-Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Central

Nervous System-AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering; DISEASE-Other Diseases

...**denaturant which is e.g. boiling- or detergent-stable and/or protease resistant protein having** chaperone **-like activity, useful for strengthening hair, nail or skin and for inducing wound healing - recombinant...**

...ABSTRACT: isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) having **chaperone** -like activity and **horseradish peroxidase** (HRP) **protection** activity, as determined by HRP **protection** assay, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic...

... a protein extract, boiling the protein extract, collecting the soluble proteins and optionally assaying for **chaperone** -like activity of the soluble proteins and enriching or isolating the stable protein having **chaperone** -like activity; (8) isolating a gene encoding (I) from a biological source; (9) identifying a...

... nucleic acid potentially encoding (I); (11) detergent-free isolation of a protease-resistant protein having **chaperone** -like activity from a biological source, involves extracting total proteins from the biological source, to...

... protease, and isolating a protease-resistant protein, and optionally assaying the protease-resistant protein for **chaperone** -like activity; (12) a fusion protein (IV) comprising (I) fused to an additional polypeptide; (13...

... steps of (M1), recovering soluble protein fraction, and optionally assaying the protease resistant protein for **chaperone** -like activity; (17) a pharmaceutical composition (V) comprising (I) as an active ingredient and a...

... 19) increasing a specific activity of a pre-isolated (I) as determined in Units of **protecting** activity per mg protein, involves autoclaving the pre-isolated (I), or treating the pre-isolated...

... isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) has **chaperone** -like activity and **horseradish peroxidase** (HRP) **protection** activity, as determined by HRP **protection** assay, of at least 10 Units/mg protein, where the HRP **protection** assay comprises mixing (I) at different final protein concentrations at a predetermined volume with 100...

...a dilution factor of (I) at a concentration of 1 mg/ml that confers 50% **protection** of HRP activity in the HRP **protection** assay. In (I), the HRP **protection** activity is of 15000, 10000, 8000, 6000, 5500, 5000, 4500, 4000, 3500, 3000, 2500, 2000...

... penalty equals 8 and gap extension penalty equals 2. (I) Is natively an oligomer. The **chaperone** -like activity includes heat stabilization of proteins. (II) Further comprises a third polynucleotide encoding an...

... extracting, boiling, collecting, assaying, and isolating of (M1), raising antibodies recognizing the stable protein having **chaperone** -like activity and screening an expression library with the antibodies; or (b) carrying out the...

... peptide bond or a cross-linker, where (IV) has an oligomeric form. ACTIVITY - Vulnerary; Nootropic; **Neuroprotective** . No biological data given. MECHANISM OF ACTION - Prevents aggregation of aggregating proteins; Inducer of immune...

... effective amount of (I) to become in contact with the protein. (I) Is useful for **protecting** an enzyme preparation from reduction in enzymatic activity, which involves adding (I) to the enzyme preparation in an amount sufficient for **protecting** the enzyme preparation from reduction in enzymatic activity. (I) is useful for repairing at least ...

... the pellet in 10 mM Tris-hydrochloric acid. The prepared stable protein when maintained with **horseradish peroxidase** , was found to maintain its activity. (176 pages)
DESCRIPTORS: recombinant denaturant stable protease resistant, **chaperone** -like protein, **horseradish peroxidase** , prep., vector-mediated gene transfer expression in host cell, antibody, fusion protein, appl. transgenic plant...

...spongyform therapy plant Armoracia rusticana enzyme EC-1.11.1.7 crop improvement vulnerary nootropic **neuroprotective** DNA sequence protein sequence (23, 22)

8/9,K/4 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0329626 DBR Accession No.: 2004-01918 PATENT
New hybrid protein chaperone **(e.g. heat shock protein) useful for stabilizing proteins and/or protein activities, or as an agent to prevent protein aggregation, or for treating diseases involving altered protein conformations - involving vector-mediated gene transfer and expression in host cell for use in gene therapy**
AUTHOR: QUINLAN R
PATENT ASSIGNEE: UNIV DUNDEE 2003
PATENT NUMBER: WO 200391266 PATENT DATE: 20031106 WPI ACCESSION NO.: 2003-865571 (200380)
PRIORITY APPLIC. NO.: GB 20029334 APPLIC. DATE: 20020423
NATIONAL APPLIC. NO.: WO 2003GB1721 APPLIC. DATE: 20030423
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A hybrid protein **chaperone** for stabilizing proteins and/or protein activities, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a stabilized protein formulation comprising at least one protein associated with the above hybrid protein **chaperone** ; (2) a method for stabilizing proteins and protein stabilities in an aqueous solution, comprising adding the above hybrid protein **chaperone** to the aqueous solution; (3) a method for stabilizing insulin in an aqueous solution, comprising adding HSP17.5, alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 to the solution; (4) a method for stabilizing citrate synthase in an aqueous solution, comprising adding alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 to the solution; (5) a method for stabilizing luciferase in an aqueous solution, comprising adding HSP17.5, alpha-crystallin or HSP27 to the

solution; (6) a method for stabilizing **horseradish peroxidase** (HRP) conjugate in an aqueous solution, comprising adding alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 to the solution; (7) a method for stabilizing an antibody or its fragment or conjugate in an aqueous solution, comprising adding HSP27 to the aqueous solution; (8) a method for stabilizing an expressed recombinant protein, comprising providing a cell capable of expressing the recombinant protein and the hybrid protein **chaperone** , and expressing the recombinant protein and the hybrid protein **chaperone** ; (9) a cell capable of expressing a recombinant protein and the hybrid protein **chaperone** ; (10) a nucleic acid sequence capable of encoding the hybrid protein **chaperone** ; and (11) a vector comprising the above nucleic acid sequence. BIOTECHNOLOGY - Preferred Protein **Chaperone** : The hybrid is a macromolecule composed of two or more portions of different origins. The portion is a region of protein or nucleic acid sequence encoding a structural domain of a protein **chaperone** or its functional homologue. The structural domain is a central domain, N- or C-terminal region of a protein **chaperone** or its functional homologue. The protein **chaperone** is a heat shock protein (HSP) selected from HSP90, HSP70 and HSP60. The protein **chaperone** is a small HSP selected from alphaA-crystallin, alphabeta-crystallin, HSP27, HSP20, MKBP, HSPB3, HSPB4, HSPB5, cvHSP, HSPB8 and HSPB9. The portions comprise sub-domains or residues of sHSP. The residue is Arginine 120. The sub-domain is C-terminal region. A potion of the **chaperone** is replaced with a similar portion from a **chaperone** of a different origin. The C-terminal portion of alphaB-crystallin is replaced with a C-terminal portion of HSP27 (alphaB-HSP27). The alphaB-HSP27 comprises the N-terminus and central portion of alphaB-crystallin and C-terminal tail of HSP27. Preferred Formulation: The ratio of protein to hybrid protein **chaperone** in the formulation is in the region of 25:1 to 1:100 (preferably 1:0.0625 to 1:40). Preferred Method: In stabilizing proteins and protein stabilities in an aqueous solution, the protein to be stabilized is an enzyme, therapeutic protein, diagnostic protein, antibody, antibody fragment or antibody conjugate. The protein is homocysteine desulphurase. The antibody conjugate is linked to an enzyme reporter. The enzyme reporter is HRP, alkaline phosphatase (ALP) or luciferase. The stabilizing is the prevention or arresting of the unfolding process and preservation of protein activity/function. The preservation of protein activity/function is achieved by assisting proteins to fold correctly and maintaining the proteins in a folded conformation. The hybrid protein **chaperone** is alphaB-crystallin and the protein is luciferase. The **chaperone** is alphaB-HSP27 and the protein is insulin, HRP conjugate or luciferase. In stabilizing insulin in an aqueous solution, the HSP17.5 is used to stabilize insulin at 37degreesC, and the HSP27 or alpha-crystallin is used to stabilize insulin at 44degreesC. In stabilizing citrate synthase in an aqueous solution, the alpha-crystallin is used to stabilize citrate synthase at 50degreesC. In stabilizing luciferase in an aqueous solution, the HSP17.5 or alphaB-crystallin is used to stabilize luciferase at room temperature. In stabilizing HRP conjugate in an aqueous solution, the HSP27 and HSP25 are used to stabilize HRP conjugate at room temperature. Alternatively, the HSP27, alphaB-crystallin or HSP25 is used to stabilize HRP conjugate at 37degreesC. In stabilizing an antibody or its fragment or conjugate in an aqueous solution, the HSP27 is used to stabilize an antibody at room temperature.Preferred Vector: The vector further comprises a nucleic acid capable of encoding a recombinant protein intended to be stabilized by the hybrid protein

chaperone . The recombinant protein is a therapeutically important protein. ACTIVITY - Cardiant; Ophthalmological; **Neuroprotective** . No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The hybrid protein **chaperone** is useful as an agent to prevent protein aggregation, as an inhibitor of cell death and genome stability pathways, for identification of proteins that are in the process of unfolding, for the treatment of diseases involving altered protein conformations (e.g. cardiomyopathies, cataract or neurodegenerative disease), or for the manufacture of a medicament for the treatment of the diseases mentioned above. The HSP17.5, alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 is used for stabilizing insulin, citrate synthase or HRP conjugate. The HSP17.5, alpha-crystallin or HSP27 may also be used for stabilizing luciferase. In addition, HSP27 may be used for stabilizing an antibody or its fragment or conjugate (all claimed). (45 pages)

DESCRIPTORS: hybrid recombinant protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. cardiomyopathy, neurodegenerative disease therapy, gene therapy DNA sequence protein sequence (23, 04)

SECTION: THERAPEUTICS-Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Cardiovascular-DISEASE-Central Nervous System; THERAPEUTICS-Gene Therapy

New hybrid protein chaperone **(e.g. heat shock protein) useful for stabilizing proteins and/or protein activities, or as...**

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A hybrid protein **chaperone** for stabilizing proteins and/or protein activities, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also...

... a stabilized protein formulation comprising at least one protein associated with the above hybrid protein **chaperone** ; (2) a method for stabilizing proteins and protein stabilities in an aqueous solution, comprising adding the above hybrid protein **chaperone** to the aqueous solution; (3) a method for stabilizing insulin in an aqueous solution, comprising...

... adding HSP17.5, alpha-crystallin or HSP27 to the solution; (6) a method for stabilizing **horseradish peroxidase** (HRP) conjugate in an aqueous solution, comprising adding alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin...

... protein, comprising providing a cell capable of expressing the recombinant protein and the hybrid protein **chaperone** , and expressing the recombinant protein and the hybrid protein **chaperone** ; (9) a cell capable of expressing a recombinant protein and the hybrid protein **chaperone** ; (10) a nucleic acid sequence capable of encoding the hybrid protein **chaperone** ; and (11) a vector comprising the above nucleic acid sequence. BIOTECHNOLOGY - Preferred Protein **Chaperone** : The hybrid is a macromolecule composed of two or more portions of different origins. The...

... a region of protein or nucleic acid sequence encoding a structural domain of a protein **chaperone** or its functional homologue. The structural domain is a central domain, N- or C-terminal region of a protein **chaperone** or its functional homologue. The protein **chaperone** is a heat shock protein (HSP) selected from HSP90, HSP70 and HSP60. The

protein **chaperone** is a small HSP selected from alphaA-crystallin, alphabeta-crystallin, HSP27, HSP20, MKBP, HSPB3, HSPB4...

... residue is Arginine 120. The sub-domain is C-terminal region. A potion of the **chaperone** is replaced with a similar portion from a **chaperone** of a different origin. The C-terminal portion of alphaB-crystallin is replaced with a...

... and C-terminal tail of HSP27. Preferred Formulation: The ratio of protein to hybrid protein **chaperone** in the formulation is in the region of 25:1 to 1:100 (preferably 1...

... proteins to fold correctly and maintaining the proteins in a folded conformation. The hybrid protein **chaperone** is alphaB-crystallin and the protein is luciferase. The **chaperone** is alphaB-HSP27 and the protein is insulin, HRP conjugate or luciferase. In stabilizing insulin ...

...acid capable of encoding a recombinant protein intended to be stabilized by the hybrid protein **chaperone** . The recombinant protein is a therapeutically important protein. ACTIVITY - Cardiant; Ophthalmological; **Neuroprotective** . No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The hybrid protein **chaperone** is useful as an agent to prevent protein aggregation, as an inhibitor of cell death...

```
? s s1(s)HRP
          59277  S1
          51633  HRP
     S10    113  S1(S)HRP
? t s6/9,k/all
```

6/9,K/1 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

18912908 BIOSIS NO.: 200600258303

Functional specificity of the mammalian Beclin-Vps34 Pl 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking

AUTHOR: Zeng Xuehuo; Overmeyer Jean H; Maltese William A (Reprint)
AUTHOR ADDRESS: Med Univ Ohio, Dept Biochem and Canc Biol, Toledo, OH 43614 USA**USA
AUTHOR E-MAIL ADDRESS: wmaltese@meduchio.edu
JOURNAL: Journal of Cell Science 119 (2): p259-270 JAN 15 2006 2006
ISSN: 0021-9533
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Beclin 1 was originally identified as a novel Bcl-2-interacting protein, but co-immunoprecipitation studies suggest that the major physiological partner for Beclin 1 is the mammalian class III phosphatidylinositol 3-kinase (PI 3-kinase) Vps34. Beclin I has been proposed to function as a tumor suppressor by promoting cellular macroautophagy, a process that is known to depend on Vps34. However, an alternative role for Beclin I in modulating normal Vps34-dependent protein trafficking pathways has not been ruled out. This possibility was examined in U-251 glioblastoma cells. Immunoprecipitates of endogenous

Beclin I contained human Vps34 (hVps34), but not Bcl-2. Suppression of Beclin 1 expression by short interfering (si)RNA-mediated gene silencing blunted the autophagic response of the cells to nutrient deprivation or C-2-ceramide. However, other PI 3-kinase-dependent trafficking pathways, such as the post-endocytic sorting of the epidermal growth factor receptor (EGFR) or the proteolytic processing of procathepsin D en route from the trans-Golgi network (TGN) to lysosomes, were not affected. Depletion of Beclin I did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin I functions mainly to engage hVps34 in the autophagic pathway.

REGISTRY NUMBERS: 9003-99-0: **horseradish peroxidase** ; 86921-29-1: procathepsin D
ENZYME COMMISSION NUMBER: EC 1.11.1.7: **horseradish peroxidase** ; EC 2.7.1.137: Vps34p
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology--Biochemistry and Molecular Biophysics; Membranes--Cell Biology
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: U-251 cell line (Hominidae)--human glioblastoma cells
COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates; Vertebrates
CHEMICALS & BIOCHEMICALS: Bcl-2; epidermal growth factor receptor; **horseradish peroxidase** ; Vps34p; procathepsin D; beclin-1
MISCELLANEOUS TERMS: endocytosis; vesicular trafficking; cellular macroautophagy
CONCEPT CODES:
02508 Cytology - Human
10064 Biochemistry studies - Proteins, peptides and amino acids
10508 Biophysics - Membrane phenomena
10802 Enzymes - General and comparative studies: coenzymes
17002 Endocrine - General
BIOSYSTEMATIC CODES:
86215 Hominidae

...ABSTRACT: affected. Depletion of Beclin I did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function...

...hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin...
...REGISTRY NUMBERS: **horseradish peroxidase** ;
...ENZYME COMMISSION NUMBER: **horseradish peroxidase** ;
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ... **horseradish peroxidase** ;

6/9,K/2 (Item 2 from file: 5)

18891097 BIOSIS NO.: 200600236492

Development and optimization of a useful assay for determining Hsp90's inherent ATPase activity

AUTHOR: Avila Christopher; Kornilayev Boris A; Blagg Brian S J (Reprint)
AUTHOR ADDRESS: Univ Kansas, Dept Med Chem, 1251 Wescoe Hall Dr,Malott 4070, Lawrence, KS 66045 USA**USA
AUTHOR E-MAIL ADDRESS: bblagg@ku.edu

JOURNAL: Bioorganic & Medicinal Chemistry 14 (4): p1134-1142 FEB 15 2006 2006
ISSN: 0968-0896
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced. (c) 2005 Elsevier Ltd. All rights reserved.

REGISTRY NUMBERS: 9003-99-0: **horseradish peroxidase** ; 9000-83-3: ATPase; 9001-37-0: glucose oxidase; 9030-19-7: maltose phosphorylase
ENZYME COMMISSION NUMBER: EC 1.11.1.7: **horseradish peroxidase** ; EC 1.1.3.4: glucose oxidase; EC 2.4.1.8: maltose phosphorylase
DESCRIPTORS:
MAJOR CONCEPTS: Pharmaceuticals--Pharmacology; Enzymology--Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: human (Hominidae)
COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates; Vertebrates
CHEMICALS & BIOCHEMICALS: **horseradish peroxidase** ; ATPase; glucose oxidase; heat shock protein 90; maltose phosphorylase
METHODS & EQUIPMENT: assay development--laboratory techniques, bioassay techniques
CONCEPT CODES:
10802 Enzymes - General and comparative studies: coenzymes
12512 Pathology - Therapy
22002 Pharmacology - General
BIOSYSTEMATIC CODES:
86215 Hominidae

ABSTRACT: The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...

...optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of

0...
...REGISTRY NUMBERS: **horseradish peroxidase** ;
...ENZYME COMMISSION NUMBER: **horseradish peroxidase** ;
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: **horseradish peroxidase** ;

6/9,K/3 (Item 3 from file: 5)

17403150 BIOSIS NO.: 200300361869
A novel chaperone **to stabilize** Horseradish Peroxidase **(HRP) based conjugates in ELISA products: An alternate plant protein to Bovine Serum Albumin (BSA).**
AUTHOR: Mehra M (Reprint); DeAnda A (Reprint); Draviam E (Reprint); Ponni Y ; Wolf A; Wang W; Shosegov O; Altman A
AUTHOR ADDRESS: Biotecx, Houston, TX, USA**USA
JOURNAL: Clinical Chemistry 49 (S6): pA117 June 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 55th Annual Meeting of the AACC (American Association for Clinical Chemistry) Philadelphia, PA, USA July 20-24, 2003; 20030720
SPONSOR: American Association for Clinical Chemistry
ISSN: 0009-9147
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English
REGISTRY NUMBERS: 67-64-1: acetone; 50-01-1: guanidine hydrochloride; 9003-99-0: **horseradish peroxidase** ; 39450-01-6: proteinase K; 9002-07-7: trypsin
ENZYME COMMISSION NUMBER: EC 1.11.1.7: **horseradish peroxidase** ; EC 3.4.21.64: proteinase K; EC 3.4.21.4: trypsin
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Salicaceae--Dicotyledones, Angiospermae, Spermatophyta, Plantae
ORGANISMS: Escherichia coli (Enterobacteriaceae)--expression system; Populus tremula {aspen tree} (Salicaceae)
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Angiosperms ; Dicots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: SDS; SP1-- **chaperone** -like protein; acetone; bovine serum albumin {BSA}; guanidine hydrochloride; **horseradish peroxidase** --conjugates; phosphate-buffered saline; protein; proteinase K; trypsin
METHODS & EQUIPMENT: Biotecx OptiCoat enzyme immunoassay kit {Biotecx OptiCoat EIA kit}--laboratory kit; ELISA--immunologic techniques, laboratory techniques; Toxoplasma IgG ELISA kit {Toxoplasma immunoglobulin G ELISA kit}--laboratory kit; enzyme immunoassay {EIA}--immunologic techniques, laboratory techniques
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10802 Enzymes - General and comparative studies: coenzymes
31000 Physiology and biochemistry of bacteria
51522 Plant physiology - Chemical constituents

BIOSYSTEMATIC CODES:
  06702 Enterobacteriaceae
  26695 Salicaceae

A novel chaperone **to stabilize** Horseradish Peroxidase **(HRP) based conjugates in ELISA products: An alternate plant protein to Bovine Serum Albumin (BSA).**
...REGISTRY NUMBERS: **horseradish peroxidase ;**
...ENZYME COMMISSION NUMBER: **horseradish peroxidase ;**
DESCRIPTORS:
  CHEMICALS & BIOCHEMICALS: ... **chaperone** -like protein...

... **horseradish peroxidase** --

6/9,K/4 (Item 4 from file: 5)

17244450 BIOSIS NO.: 200300203169
Stabilization of heat-induced changes in plant peroxidase preparations by ClpX, a bacterial heat shock protein.
AUTHOR: Kroczynska Barbara (Reprint); Ciesielski Arkadiusz; Sergio Lucrezia
AUTHOR ADDRESS: Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 900 South Ashland Avenue, Molecular Biology Research Building, Chicago, IL, 60607-7173, USA**USA
AUTHOR E-MAIL ADDRESS: kroczyb@uic.edu
JOURNAL: Journal of Plant Physiology 159 (12): p1295-1299 December 2002 2002
MEDIUM: print
ISSN: 0176-1617
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Peroxidases (PODs) are known to be quite stable at elevated temperatures. Moreover, partially denatured peroxidases are able to regain their catalytic activity during incubation at room temperature. In this paper, we describe the effects of some heat shock proteins on the self-reactivation of plant peroxidase preparations. Horseradish and artichoke peroxidases (HRP and ARP, respectively) were first heated (at 60 degreeC or 90 degreeC), then incubated at a slightly elevated temperature (30 degreeC). The heat-treatment resulted in a considerable loss of activity of both enzymes but the subsequent incubation allowed their reactivation. However, no reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding that would result in aggregation.

REGISTRY NUMBERS: 9003-99-0: peroxidase
ENZYME COMMISSION NUMBER: EC 1.11.1.7: peroxidase
DESCRIPTORS:
  MAJOR CONCEPTS: Enzymology--Biochemistry and Molecular Biophysics
  BIOSYSTEMATIC NAMES: Enterobacteriaceae--Facultatively Anaerobic

Gram-Negative Rods, Eubacteria, Bacteria, Microorganisms; Compositae--Dicotyledones, Angiospermae, Spermatophyta, Plantae; Cruciferae--Dicotyledones, Angiospermae, Spermatophyta, Plantae
ORGANISMS: Escherichia coli (Enterobacteriaceae); Cynara cardunculus var. scolymus {artichoke} (Compositae); horseradish (Cruciferae)
COMMON TAXONOMIC TERMS: Bacteria; Eubacteria; Microorganisms; Angiosperms; Dicots; Plants; Spermatophytes; Vascular Plants
CHEMICALS & BIOCHEMICALS: peroxidase--stabilization, heat-induced change; ClpX--bacterial heat shock protein; protein--refolding
METHODS & EQUIPMENT: electrophoresis--electrophoretic techniques, laboratory techniques
CONCEPT CODES:
10064 Biochemistry studies - Proteins, peptides and amino acids
10802 Enzymes - General and comparative studies: coenzymes
31000 Physiology and biochemistry of bacteria
51518 Plant physiology - Enzymes
BIOSYSTEMATIC CODES:
06702 Enterobacteriaceae
25840 Compositae
25880 Cruciferae

...ABSTRACT: reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding...

6/9,K/5 (Item 5 from file: 5)

17231877 BIOSIS NO.: 200300190596
Phospholipid assisted folding of a denatured heme protein: Effect of phosphatidylethanolamine.
AUTHOR: Debnath Dilip; Bhattacharya Shekhar; Chakrabarti Abhijit (Reprint)
AUTHOR ADDRESS: Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Kolkata, 700037, India**India
AUTHOR E-MAIL ADDRESS: abhijit@biop.saha.ernet.in
JOURNAL: Biochemical and Biophysical Research Communications 301 (4): p 979-984 February 21, 2003 2003
MEDIUM: print
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The role of the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins have not been done so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its refolding from denatured state. Dimyristoylphosphatidylethanolamine (DMPE), a bilayer-forming PE, was able to increase the reactivation yield of denatured HRP upon 30 min

refolding at 25degreeC. However, dioleoylphosphatidylethanolamine (DOPE), containing one double bond in the fatty acid chains, which does not favour bilayer organization, did not support proper refolding. The phospholipids with N-methylated head groups, phosphatidylcholines, e.g., DMPC and DOPC showed differential effects when DMPC remained mostly non-supportive while DOPC on the contrary led to inhibition of the refolding of the denatured heme enzyme. Fluorescence spectroscopic studies also indicated changes in the microenvironments of the heme moiety and the single tryptophan residue of HRP in presence of the aminophospholipid.

REGISTRY NUMBERS: 9003-99-0: **horseradish peroxidase** ; 20255-95-2: dimyristoylphosphatidylethanolamine; 2462-63-7Q: dioleoylphosphatidylethanolamine; 4004-05-1Q: dioleoylphosphatidylethanolamine; 68737-67-7: dioleoylphosphatidylcholine; 18194-24-6Q: dimyristoylphosphatidylcholine; 18656-38-7Q: dimyristoylphosphatidylcholine
ENZYME COMMISSION NUMBER: EC 1.11.1.7: **horseradish peroxidase**
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
CHEMICALS & BIOCHEMICALS: phosphatidylethanolamine--non-protein molecular **chaperone** ; **horseradish peroxidase** --denatured state, phospholipid assisted folding; dimyristoylphosphatidylethanolamine; dioleoylphosphatidylethanolamine; dioleoylphosphatidylcholine; dimyristoylphosphatidylcholine
METHODS & EQUIPMENT: fluorescence spectroscopy--laboratory techniques, spectrum analysis techniques
MISCELLANEOUS TERMS: protein folding
CONCEPT CODES:
10060 Biochemistry studies - General
10066 Biochemistry studies - Lipids
10802 Enzymes - General and comparative studies: coenzymes

...ABSTRACT: the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins...

...so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its...
...REGISTRY NUMBERS: **horseradish peroxidase** ;
...ENZYME COMMISSION NUMBER: **horseradish peroxidase**
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...non-protein molecular **chaperone** ; ...

... **horseradish peroxidase** --

6/9,K/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17221860 BIOSIS NO.: 200300180579
Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone **function.**

AUTHOR: Shin Bong Kyung; Wang Hong; Yim Anne Marie; Le Naour Francois; Brichory Franck; Jang Jun Ho; Zhao Rong; Puravs Eric; Tra John; Michael Claire W; Misek David E (Reprint); Hanash Samir M
AUTHOR ADDRESS: Dept. of Pediatrics, University of Michigan, 1150 West Medical Center Dr., Rm. A520 MSRB-1, Ann Arbor, MI, 48109-0656, USA**USA
AUTHOR E-MAIL ADDRESS: dmisek@umich.edu
JOURNAL: Journal of Biological Chemistry 278 (9): p7607-7616 February 28, 2003 2003
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is currently limited data available pertaining to the global characterization of the cell surface proteome. We have implemented a strategy for the comprehensive profiling and identification of surface membrane proteins. This strategy has been applied to cancer cells, including the SH-SY5Y neuroblastoma, the A549 lung adenocarcinoma, the LoVo colon adenocarcinoma, and the Sup-B15 acute lymphoblastic leukemia (B cell) cell lines and ovarian tumor cells. Surface membrane proteins of viable, intact cells were subjected to biotinylation then affinity-captured and purified on monomeric avidin columns. The biotinylated proteins were eluted from the monomeric avidin columns as intact proteins and were subsequently separated by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained for the different cell populations analyzed. Identification of a subset of biotinylated proteins among the different cell populations analyzed using matrix-assisted laser desorption ionization and tandem mass spectrometry uncovered proteins with a restricted expression pattern in some cell line(s), such as CD87 and the activin receptor type IIB. We also identified more widely expressed proteins, such as CD98, and a sushi repeat-containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70, HSP60, HSP54, HSP27, and protein disulfide isomerase. Comprehensive profiling of the cell surface proteome provides an effective approach for the identification of commonly occurring proteins as well as proteins with restricted expression patterns in this compartment.

REGISTRY NUMBERS: 37318-49-3: protein disulfide isomerase; 7287-19-6: selectin
ENZYME COMMISSION NUMBER: EC 5.3.4.1: protein disulfide isomerase
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Membranes--Cell Biology; Tumor Biology
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: A549 cell line (Hominidae); SH-SY5Y cell line (Hominidae); LoVo cell line (Hominidae); Sup-B15 cell line (Hominidae); human (Hominidae)
ORGANISMS: PARTS ETC: cell surface; cell
COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates; Vertebrates

DISEASES: lung adenocarcinoma--neoplastic disease, respiratory system disease; ovarian cancer--neoplastic disease, reproductive system disease/female; neuroblastoma--neoplastic disease, nervous system disease; colon adenocarcinoma--digestive system disease, neoplastic disease
MESH TERMS: Adenocarcinoma (MeSH), Lung Neoplasms (MeSH); Ovarian Neoplasms (MeSH); Neuroblastoma (MeSH); Colonic Neoplasms (MeSH), Adenocarcinoma (MeSH)
CHEMICALS & BIOCHEMICALS: proteome; streptavidin- **horseradish peroxidase** ; GRP78; GRP75; HSP70 {heat shock protein 70}; HSP60; HSP54; HSP27; protein disulfide isomerase; CD87; activin receptor type IIB; CD98; selectin
METHODS & EQUIPMENT: avidin column--laboratory equipment; affinity capture--laboratory techniques; tandem mass spectrometry--laboratory techniques, spectrum analysis techniques; matrix-assisted laser/desorption ionization mass spectrometry--laboratory techniques, spectrum analysis techniques; two-dimensional PAGE--electrophoretic techniques, laboratory techniques; polyvinylidene difluoride membrane --laboratory equipment
CONCEPT CODES:
02508 Cytology - Human
10508 Biophysics - Membrane phenomena
10060 Biochemistry studies - General
10064 Biochemistry studies - Proteins, peptides and amino acids
10802 Enzymes - General and comparative studies: coenzymes
14006 Digestive system - Pathology
16006 Respiratory system - Pathology
16506 Reproductive system - Pathology
20506 Nervous system - Pathology
24004 Neoplasms - Pathology, clinical aspects and systemic effects
BIOSYSTEMATIC CODES:
86215 Hominidae

...**profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with** chaperone **function.**

...ABSTRACT: by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained...

...containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...streptavidin- **horseradish peroxidase** ;

6/9,K/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17206296 BIOSIS NO.: 200300165015
Glycated Proteins Become Substrates of Amadoriase Enzyme Upon Amidation of Carboxylate: Role of Charge on the Substrate Specificity of Amadoriase.
AUTHOR: Monnier V M (Reprint); Wu X (Reprint)

AUTHOR ADDRESS: Pathology and Biochemistry, Case Western Reserve Univ, Cleveland, OH, USA**USA
JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002 p Abstract No. 2384 2002 2002
MEDIUM: cd-rom
CONFERENCE/MEETING: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002; 20020505
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Purpose: Glycation of lens crystallins increase their susceptibility toward oxidation and crosslinking during aging. Glycated crystallins also have impaired **chaperone** activity. When combined these processes may greatly increase the predisposition of the lens toward cataractogenesis. To counter the effects of excessive glycation, we are developing genetic and pharmacological strategies, among which amadoriase enzymes that can reverse the glycation process. Amadoriase is a deglycation enzyme that oxidizes glycated substrate and generates glucosone, H2O2 and free amine group. However, amadoriase so far only recognizes small molecules as substrates and cannot deglycate large protein such as glycated BSA. We report the unexpected finding that glycated proteins and lens crystallins can become substrates of amadoriase upon amidation of the carboxylate residues. Method: Glycated BSA as model protein and bovine alpha-crystallins were amidated by reacting the protein with NH4Cl using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as coupling reagent. Deglycation activity was measured in terms of H2O2 release using amplex red and **horseradish peroxidase** . Result: Incubation of the substrate with recombinant Amadoriase I from Aspergillus sp. led to progressive formation of H2O2 and release of glucosone with no detectable activity against non-amidated glycated BSA or crystallins. The extent of deglycation of lysine residues (furosine method) confirmed that around 10% of the glycation sites on the protein were deglycated. Further studies with affinity purified albumin from diabetic patient serum revealed in vivo glycated proteins can also become substrates of amadoriase as a function of degree of modification. CD spectra of BSA, glycated BSA and amidated glycated BSA showed no major conformational changes after amidation. Negative ions such as PO4-3 and SO4-2 also inhibited the activity, while esterifying glycated BSA, which also removes negative charges, restored the activity. The sites of deglycation on albumin and crystallins are under investigation by mass spectrometry. Conclusion: These results suggest not only steric hindrance, but electrostatic repulsion between the negative charges of the enzyme-protein substrate complex can selectively affect substrate accessibility. This discovery provides clues for the future engineering of amadoriases with the new activity towards glycated protein. By extrapolation, these results suggest the age-related deamidation of lens crystallins may alter the behavior of lens crystallins as substrate toward certain enzymes.

REGISTRY NUMBERS: 153302-42-2: amadoriase; 1892-57-5: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; 19746-33-9: furosine
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Sense Organs--Sensory Reception

CHEMICALS & BIOCHEMICALS: glycated proteins; amadoriase enzyme; carboxylate; amadoriase; glycated crystallins; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide {EDC}; furosine
METHODS & EQUIPMENT: mass spectrometry--laboratory techniques, spectrum analysis techniques
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10060 Biochemistry studies - General
20004 Sense organs - Physiology and biochemistry

...ABSTRACT: crystallins increase their susceptibility toward oxidation and crosslinking during aging. Glycated crystallins also have impaired **chaperone** activity. When combined these processes may greatly increase the predisposition of the lens toward cataractogenesis...

...coupling reagent. Deglycation activity was measured in terms of H2O2 release using amplex red and **horseradish peroxidase** . Result: Incubation of the substrate with recombinant Amadoriase I from Aspergillus sp. led to progressive...

6/9,K/8 (Item 8 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

17184730 BIOSIS NO.: 200300143449
Distribution of Heme Oxygenase-1 (HO-1) in Rabbit Cornea.
AUTHOR: Chang R I (Reprint); McCanna D; Kalsow C M
AUTHOR ADDRESS: Ophthalmology, University Rochester, Rochester, NY, USA** USA
JOURNAL: ARVO Annual Meeting Abstract Search and Program Planner 2002 p Abstract No. 1678 2002 2002
MEDIUM: cd-rom
CONFERENCE/MEETING: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002; 20020505
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Purpose: Heme oxygenase-1 (HO-1) also named heat shock protein 32 (Hsp32) is a molecular **chaperone** protein that can be induced in response to increased oxidative stress, hypoxia, heme, metals or inflammatory cytokines. Upregulation of HO-1 mRNA and protein have been measured in rabbit corneal epithelia in response to heme, metals, and inflammatory cytokines as well as hypoxia and oxidative stress (Biochem. Pharmacol. 53:1069, 1997). Homogenization of tissue for these quantitative studies precludes any localization of HO-1. The current immunohistochemical study demonstrates the distribution of HO-1 in rabbit cornea. Method: Corneas of 10 New Zealand white rabbits (2.5-3.5kg) were fixed in 95% ethanol and embedded in paraffin. HO-1 was detected using mouse anti-heme-oxygenase-1 monoclonal antibody (MAb HO-1) as the primary antibody. Distribution of immunoreactivity was visualized with a 1horseradish peroxidase mouse Envision+ System with DAB substrate. Specificity of reactivity was confirmed by comparison of reactivity patterns for other heat shock proteins, Hsp 47 and inducible and constitutive Hsp70. Results: HO-1 immunoreactivity in the corneal

epithelium was primarily observed in the cytoplasm of basal epithelial cells. The reactivity throughout the corneal epithelium was not uniform, i.e., some areas had no reactivity while other areas had reactivity throughout the epithelium including wing and superficial cells. MAb HO-1 reacted consistently and uniformly with limbal vascular endothelium. The epithelial and endothelial patterns of reactivity with MAb HO-1 were distinct from those of the other hsp's tested. Conclusion: These experiments qualitatively demonstrate the distribution of HO-1 in normal rabbit cornea. The variability of expression in different areas of the corneal epithelium indicates the responsive nature of HO-1 expression to changes in the oxidative state of the microenvironment. Communication between the different layers of epithelium and the change in molecular machinery as cells mature from the basal layer to the more superficial area could play a role in the rapid response. Since corneal epithelium and limbal vascular endothelium are at the interface of the cornea with the external environment and the internal (vascular) environment of the cornea, HO-1 is a potentially important protective mechanism for this tissue. HO-1 expression may be exploited as an early indicator of corneal oxidative stress and/or as a means to enhance protection from oxidative stress. Support: Rochester Eye and Human Parts Bank and Bausch and Lomb, Inc.

REGISTRY NUMBERS: 9003-99-0: **horseradish peroxidase**
ENZYME COMMISSION NUMBER: EC 1.11.1.7: **horseradish peroxidase**
DESCRIPTORS:
MAJOR CONCEPTS: Enzymology--Biochemistry and Molecular Biophysics; Sense Organs--Sensory Reception
BIOSYSTEMATIC NAMES: Leporidae--Lagomorpha, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: rabbit (Leporidae); New Zealand white rabbit (Leporidae)
ORGANISMS: PARTS ETC: cornea--sensory system; corneal epithelium--sensory system
COMMON TAXONOMIC TERMS: Animals; Chordates; Lagomorphs; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Vertebrates
CHEMICALS & BIOCHEMICALS: heme oxygenase-1 {HO-1}; heat shock protein 32 {Hsp32}; cytokines; anti-heme-oxygenase-1 monoclonal antibody; **horseradish peroxidase**
METHODS & EQUIPMENT: immunohistochemical study--immunologic techniques, laboratory techniques
MISCELLANEOUS TERMS: oxidative stress; Meeting Abstract
CONCEPT CODES:
00520 General biology - Symposia, transactions and proceedings
10802 Enzymes - General and comparative studies: coenzymes
20004 Sense organs - Physiology and biochemistry
BIOSYSTEMATIC CODES:
86040 Leporidae

...ABSTRACT: Heme oxygenase-1 (HO-1) also named heat shock protein 32 (Hsp32) is a molecular **chaperone** protein that can be induced in response to increased oxidative stress, hypoxia, heme, metals or...

...antibody (MAb HO-1) as the primary antibody. Distribution of immunoreactivity was visualized with a **horseradish peroxidase** mouse Envision+ System with DAB substrate. Specificity of reactivity was confirmed by comparison of reactivity...
...REGISTRY NUMBERS: **horseradish peroxidase**
...ENZYME COMMISSION NUMBER: **horseradish peroxidase**

DESCRIPTORS:
  CHEMICALS & BIOCHEMICALS: ... **horseradish peroxidase**

6/9,K/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

16072712 BIOSIS NO.: 200100244551
Binding of a denatured heme protein and ATP to erythroid spectrin
AUTHOR: Chakrabarti Abhijit (Reprint); Bhattacharya Shekhar; Ray Sibnath; Bhattacharyya Malyasri
AUTHOR ADDRESS: Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta, 700037, India**India
JOURNAL: Biochemical and Biophysical Research Communications 282 (5): p 1189-1193 April 20, 2001 2001
MEDIUM: print
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Spectrin is a large, worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of increasing concentrations of spectrin from that in the absence. This indicated that spectrin could bind denatured HRP and inhibit their refolding. In presence of 1 mM ATP and 10 mM MgCl2 the spectrin binding of denatured HRP is abolished. This activity of decreasing the reactivation yield was found to be ATP-dependent and the denatured enzyme after 30 min refolding in the presence of spectrin, pretreated with Mg/ATP, showed about 40% increase in the reactivation yield compared to the same in absence of spectrin. Fluorescence spectroscopic studies indicated binding of ATP to native spectrin showing concentration-dependent quenching of tryptophan fluorescence by ATP. The apparent dissociation constant of binding of ATP to spectrin was estimated to be 1.1 mM. A high affinity binding of spectrin with denatured HRP has been characterized (Kd = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes.

REGISTRY NUMBERS: 56-65-5Q: ATP; 42530-29-0Q: ATP; 94587-45-8Q: ATP; 111839-44-2Q: ATP
DESCRIPTORS:
  MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Blood and Lymphatics--Transport and Circulation
  BIOSYSTEMATIC NAMES: Bovidae--Artiodactyla, Mammalia, Vertebrata, Chordata, Animalia
  ORGANISMS: goat (Bovidae)
  ORGANISMS: PARTS ETC: erythroid cell--blood and lymphatics
  COMMON TAXONOMIC TERMS: Animals; Artiodactyls; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Vertebrates
  CHEMICALS & BIOCHEMICALS: ATP; heme protein--binding, denatured; spectrin
CONCEPT CODES:
  10062 Biochemistry studies - Nucleic acids, purines and pyrimidines

02506 Cytology - Animal
10060 Biochemistry studies - General
15002 Blood - Blood and lymph studies
15004 Blood - Blood cell studies
BIOSYSTEMATIC CODES:
85715 Bovidae

...ABSTRACT: worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of...

...been characterized (Kd = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes.

6/9,K/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

15507727 BIOSIS NO.: 200000226040
Spectrin exhibits chaperone **-like activity**
AUTHOR: Chakrabarti Abhijit (Reprint); Bhattacharya Shekhar
AUTHOR ADDRESS: Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta, 700 037, India**India
JOURNAL: Current Science (Bangalore) 77 (6): p812-813 Sept., 1999 1999
MEDIUM: print
ISSN: 0011-3891
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Erythroid spectrin, a cytoskeletal protein, inhibits refolding of denatured heme enzyme, **horseradish peroxidase** (HRP). The denatured enzyme after 30 min refolding in the absence and presence of spectrin showed significant differences in the enzyme activity. The enzymic activity of HRP decreased in the presence of spectrin when compared with the activity in absence of spectrin. This inhibitory effect of spectrin is abolished when it was preincubated with magnesium-ATP indicating that spectrin exhibits **chaperone** -like activity.
REGISTRY NUMBERS: 1476-84-2: magnesium-ATP
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
CHEMICALS & BIOCHEMICALS: **horseradish peroxidase** --activity, denatured, refolding; magnesium-ATP; spectrin--chaperonin-like activity, molecular **chaperone**
CONCEPT CODES:
10064 Biochemistry studies - Proteins, peptides and amino acids
10506 Biophysics - Molecular properties and macromolecules
10806 Enzymes - Chemical and physical
10808 Enzymes - Physiological studies
10062 Biochemistry studies - Nucleic acids, purines and pyrimidines
10069 Biochemistry studies - Minerals

Spectrin exhibits chaperone **-like activity**

ABSTRACT: Erythroid spectrin, a cytoskeletal protein, inhibits refolding of denatured heme enzyme, **horseradish peroxidase** (HRP). The denatured enzyme after 30 min refolding in the absence and presence of spectrin...

...of spectrin is abolished when it was preincubated with magnesium-ATP indicating that spectrin exhibits **chaperone** -like activity.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: **horseradish peroxidase** --...

...chaperonin-like activity, molecular **chaperone**

6/9,K/11 (Item 11 from file: 5)

15083139 BIOSIS NO.: 199900342799
Functional early endosomes are required for maturation of major histocompatibility complex class II molecules in human B lymphoblastoid cells
AUTHOR: Pond Leslie; Watts Colin (Reprint)
AUTHOR ADDRESS: Department of Biochemistry, University of Dundee, Wellcome Trust Building, Dundee, DD1 4HN, UK**UK
JOURNAL: Journal of Biological Chemistry 274 (25): p18049-18054 June 18, 1999 1999
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must be degraded to enable peptide capture by MHC class II molecules. It remains controversial exactly which route or routes MHC class II/Ii complexes take to reach the sites of Ii processing and peptide loading. We have asked whether early endosomes are required for successful maturation of MHC class II molecules by using an in situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas transport of secretory Ig through the secretory pathway is virtually normal in the ablated cells, newly synthesized MHC class II/Ii complexes never reach compartments capable of processing Ii. These results strongly suggest that the transport of the bulk of newly synthesized MHC class II molecules through early endosomes is obligatory and that direct input into later endosomes/lysosomes does not take place.

DESCRIPTORS:
MAJOR CONCEPTS: Cell Biology; Immune System--Chemical Coordination and Homeostasis
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: human (Hominidae)
ORGANISMS: PARTS ETC: B lymphoblastoid cell--blood and lymphatics

COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates; Vertebrates
CHEMICALS & BIOCHEMICALS: early endosome; major histocompatibility complex class II molecules--invariant chain, invariant chain **chaperone** , maturation
METHODS & EQUIPMENT: peroxidase-ablation technique--cytological method
CONCEPT CODES:
02508 Cytology - Human
10060 Biochemistry studies - General
34502 Immunology - General and methods
00532 General biology - Miscellaneous
BIOSYSTEMATIC CODES:
86215 Hominidae

ABSTRACT: Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must...

...situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin-**horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...invariant chain, invariant chain **chaperone** , maturation

6/9,K/12 (Item 12 from file: 5)

11815812 BIOSIS NO.: 199395118078
Organelle-specific phosphorylation: Identification of unique membrane phosphoproteins of the endoplasmic reticulum and endosomal apparatus
AUTHOR: Rindress Donna (Reprint); Lei Xia; Ahluwalia Jatinder P S; Cameron Pamela H; Fazel Ali; Posner Barry I; Bergeron John J M
AUTHOR ADDRESS: Dep. Anatomy, McGill University, Montreal, Quebec H3A 2B2, Canada**Canada
JOURNAL: Journal of Biological Chemistry 268 (7): p5139-5147 1993
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Highly purified endoplasmic reticulum fractions from rat liver and dog pancreas harbor membrane-associated kinases that phosphorylate integral membrane proteins of 90, 56, 35, and 15 kDa with (gamma-32P) GTP and of 90, 56, and 35 kDa with (gamma-32P)ATP. Of these, only the 35-kDa phosphoprotein was N-glycosylated. Screening of Golgi fractions, endosomes, plasma membranes, lysosomes, and mitochondria revealed phosphoproteins unique to each organelle. In particular, endosomes were found to harbor a 48-kDa extrinsic membrane protein and two or more integral membrane phosphoproteins of 30-35 kDa. None of these were N-glycosylated as judged by their insensitivity to digestion by N-glycosidase F and a lack of binding to concanavalin A or wheat germ agglutinin. Since the 30-35-kDa membrane phosphoproteins present in

Golgi-free endosomal fractions were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique to endosomes. Since membrane phosphoproteins unique to the endoplasmic reticulum have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P. H., Ou, W.-J., Doherty, J.-J., II, Louvard, D., Bell, A. W., Dignard, D., Thomas, D. Y., and Bergeron, J. J. M. (1991) J. Biol. Chem. 266, 19599-19610; Ahluwalia, N., Bergeron, J. J. M., Wada, I., Degen, E., and Williams, D. B. (1992) J. Biol. Chem. 267, 10914-10918), then the unique endosomal phosphoproteins may serve equally important functions in addition to serving as novel markers for the organelle.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Bioenergetics--Biochemistry and Molecular Biophysics; Cell Biology; Digestive System--Ingestion and Assimilation; Membranes--Cell Biology; Methods and Techniques
BIOSYSTEMATIC NAMES: Canidae--Carnivora, Mammalia, Vertebrata, Chordata, Animalia; Muridae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: Canidae (Canidae); Muridae (Muridae)
COMMON TAXONOMIC TERMS: Carnivores; Animals; Chordates; Mammals; Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Vertebrates
MISCELLANEOUS TERMS: ANALYTICAL METHOD; DOG PANCREAS; ENDOSOME; GOLGI APPARATUS; LYSOSOME; MITOCHONDRIA; RAT LIVER
CONCEPT CODES:
01004 Methods - Laboratory methods
02506 Cytology - Animal
10054 Biochemistry methods - Proteins, peptides and amino acids
10064 Biochemistry studies - Proteins, peptides and amino acids
10508 Biophysics - Membrane phenomena
10510 Biophysics - Bioenergetics: electron transport and oxidative phosphorylation
14004 Digestive system - Physiology and biochemistry
BIOSYSTEMATIC CODES:
85765 Canidae
86375 Muridae

...ABSTRACT: were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique...

...have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P. H., Ou, W.-J., Doherty, J.-J., II...

6/9,K/13 (Item 1 from file: 24)
DIALOG(R)File 24:CSA Life Sciences Abstracts

0002790693 IP ACCESSION NO: 6653296
Development and optimization of a useful assay for determining Hsp90's inherent ATPase activity

Avila, Christopher; Kornilayev, Boris A; Blagg, Brian SJ
The Department of Medicinal Chemistry and The Center for Protein Structure and Function, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045-7563, USA, [mailto:bblagg@ku.edu]

Bioorganic and Medicinal Chemistry, v 14, n 4, p 1134-1142, February 15, 2006
PUBLICATION DATE: 2006

PUBLISHER: Elsevier Science Ltd., The Boulevard Langford Lane Kidlington Oxford OX5 1GB UK, [mailto:usinfo-f@elsevier.com], [URL:http://www.elsevier.nl]

DOCUMENT TYPE: Journal Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English
ISSN: 0968-0896
ELECTRONIC ISSN: 1464-3391
DOI: 10.1016/j.bmc.2005.09.027
FILE SEGMENT: Medical & Pharmaceutical Biotechnology Abstracts

ABSTRACT:
The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced.

DESCRIPTORS: Hsp90 protein; Adenosinetriphosphatase; Protein folding; Glucose oxidase; Maltose phosphorylase; Signal transduction; Cancer; Chaperones; **horseradish peroxidase**
SUBJ CATG: 33243, Molecular methods
ABSTRACT:
The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...

...optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0 ...

DESCRIPTORS: Hsp90 protein; Adenosinetriphosphatase; Protein folding; Glucose oxidase; Maltose phosphorylase; Signal transduction; Cancer; Chaperones; **horseradish peroxidase**

6/9,K/14 (Item 2 from file: 24)
DIALOG(R)File 24:CSA Life Sciences Abstracts

0001979952 IP ACCESSION NO: 4534443

Functional Early Endosomes Are Required for Maturation of Major Histocompatibility Complex Class II Molecules in Human B Lymphoblastoid Cells

Pond, L; Watts, C
Department of Biochemistry, Wellcome Trust Building, University of Dundee, Dundee DD1 4HN, UK

Journal of Biological Chemistry, v 274, n 25, p 18049-18054, June 18, 1999
PUBLICATION DATE: 1999

DOCUMENT TYPE: Journal Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English
ISSN: 0021-9258
FILE SEGMENT: Immunology Abstracts

ABSTRACT:
Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must be degraded to enable peptide capture by MHC class II molecules. It remains controversial exactly which route or routes MHC class II/Ii complexes take to reach the sites of Ii processing and peptide loading. We have asked whether early endosomes are required for successful maturation of MHC class II molecules by using an in situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas transport of secretory Ig through the secretory pathway is virtually normal in the ablated cells, newly synthesized MHC class II/Ii complexes never reach compartments capable of processing Ii. These results strongly suggest that the transport of the bulk of newly synthesized MHC class II molecules through early endosomes is obligatory and that direct input into later endosomes/lysosomes does not take place.

DESCRIPTORS: Lymphoblastoid cell lines; Lymphocytes B; Major histocompatibility complex; Lysosomes; endosomes
IDENTIFIERS: man; class II molecules
SUBJ CATG: 06749, Function

ABSTRACT:
Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must...

...situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas...

6/9,K/15 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

14838954 Genuine Article#: 012HV Number of References: 86

Title: Functional specificity of the mammalian Beclin-Vps34 Pl 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking

Author(s): Zeng XH; Overmeyer JH; Maltese WA (REPRINT)
Corporate Source: Med Univ Ohio,Dept Biochem & Canc Biol,Toledo//OH/43614 (REPRINT); Med Univ Ohio,Dept Biochem & Canc Biol,Toledo//OH/43614(wmaltese@meduchio.edu)

Journal: JOURNAL OF CELL SCIENCE, 2006, V119, N2 (JAN 15), P259-270
ISSN: 0021-9533 Publication date: 20060115
Publisher: COMPANY OF BIOLOGISTS LTD, BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND
Language: English Document Type: ARTICLE
Geographic Location: USA
Journal Subject Category: CELL BIOLOGY

Abstract: Beclin 1 was originally identified as a novel Bcl-2-interacting protein, but co-immunoprecipitation studies suggest that the major physiological partner for Beclin 1 is the mammalian class III phosphatidylinositol 3-kinase (PI 3-kinase) Vps34. Beclin I has been proposed to function as a tumor suppressor by promoting cellular macroautophagy, a process that is known to depend on Vps34. However, an alternative role for Beclin I in modulating normal Vps34-dependent protein trafficking pathways has not been ruled out. This possibility was examined in U-251 glioblastoma cells. Immunoprecipitates of endogenous Beclin I contained human Vps34 (hVps34), but not Bcl-2. Suppression of Beclin 1 expression by short interfering (si)RNA-mediated gene silencing blunted the autophagic response of the cells to nutrient deprivation or C-2-ceramide. However, other PI 3-kinase-dependent trafficking pathways, such as the post-endocytic sorting of the epidermal growth factor receptor (EGFR) or the proteolytic processing of procathepsin D en route from the trans-Golgi network (TGN) to lysosomes, were not affected. Depletion of Beclin I did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin I functions mainly to engage hVps34 in the autophagic pathway.

Descriptors--Author Keywords: Beclin ; autophagy ; Vps34 ; phosphatidylinositol-3-kinase ; cell death ; endocytosis ; glioblastoma
Identifiers--KeyWord Plus(R): MALIGNANT GLIOMA-CELLS; MULTIVESICULAR BODY MORPHOGENESIS; PHOSPHATIDYLINOSITOL 3-KINASE; TUMOR-SUPPRESSOR; CATHEPSIN-D; SACCHAROMYCES-CEREVISIAE; MOLECULAR-MECHANISMS; MEMBRANE-TRANSPORT; AUTOPHAGIC VACUOLE; RAT HEPATOCYTES

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TANIDA I, 2002, V277, P13739, J BIOL CHEM
TANIDA I, 2004, V36, P2503, INT J BIOCHEM CELL B
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...Abstract: affected. Depletion of Beclin I did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function...

...hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin...

6/9,K/16 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

14781357 Genuine Article#: 006PP Number of References: 42
Title: Development and optimization of a useful assay for determining Hsp90's inherent ATPase activity
Author(s): Avila C; Kornilayev BA; Blagg BSJ (REPRINT)
Corporate Source: Univ Kansas,Dept Med Chem,1251 Wescoe Hall Dr,Malott 4070/Lawrence//KS/66045 (REPRINT); Univ Kansas,Dept Med Chem,Lawrence//KS/66045; Univ Kansas,Ctr Prot Struct & Funct,Lawrence//KS/66045; Univ Kansas,Biochem Res Serv Lab,Lawrence//KS/66047(bblagg@ku.edu)
Journal: BIOORGANIC & MEDICINAL CHEMISTRY, 2006, V14, N4 (FEB 15), P 1134-1142
ISSN: 0968-0896 Publication date: 20060215
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND
Language: English Document Type: ARTICLE
Geographic Location: USA
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; CHEMISTRY, MEDICINAL; CHEMISTRY, ORGANIC
Abstract: The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling

cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced. (c) 2005 Elsevier Ltd. All rights reserved.

Descriptors--Author Keywords: Hsp90 ; inhibitors ; assay development ; cancer

Identifiers--KeyWord Plus(R): AMINO-TERMINAL DOMAIN; MOLECULAR **CHAPERONE** ; NUCLEOTIDE-BINDING; IN-VIVO; GELDANAMYCIN; RADICICOL; INHIBITION; PROTEINS; TARGET; DERIVATIVES

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Abstract: The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...

...optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0...
...Identifiers--AMINO-TERMINAL DOMAIN; MOLECULAR **CHAPERONE** ; NUCLEOTIDE-BINDING; IN-VIVO; GELDANAMYCIN; RADICICOL; INHIBITION; PROTEINS; TARGET; DERIVATIVES

6/9,K/17 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

12070198 Genuine Article#: 682JP Number of References: 0
Title: A novel chaperone **to stabilize** Horseradish Peroxidase **(HRP) based conjugates in ELISA products: An alternate plant protein to Bovine Serum Albumin (BSA).**
Author(s): Mehra M; DeAnda A; Draviam E; Ponni Y; Wolf A; Wang W; Shosegov O; Altman A
Corporate Source: Biotecx,Houston//TX/; Fulcrum SP Ltd,Herzliah//Israel/; Hebrew Univ Jerusalem,Jerusalem//Israel/
Journal: CLINICAL CHEMISTRY, 2003, V49, N6,2,S (JUN), PA117-A117
ISSN: 0009-9147 Publication date: 20030600
Publisher: AMER ASSOC CLINICAL CHEMISTRY, 2101 L STREET NW, SUITE 202, WASHINGTON, DC 20037-1526 USA
Language: English Document Type: MEETING ABSTRACT
Geographic Location: USA; Israel
Journal Subject Category: MEDICAL LABORATORY TECHNOLOGY

Title: A novel chaperone **to stabilize** Horseradish Peroxidase **(HRP) based conjugates in ELISA products: An alternate plant protein to Bovine Serum Albumin (BSA).**

6/9,K/18 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

12031668 Genuine Article#: 724ZM Number of References: 29
Title: Studies of peroxidase refolding in the presence of specific antibodies
Author(s): Bezsudnova EY (REPRINT) ; Zherdev AV; Ermolenko DN; Yakovleva IV ; Sviridov VV; Popov VO; Dzantiev BB
Corporate Source: Russian Acad Sci,Bach Inst Biochem,Moscow 119071//Russia/ (REPRINT); Russian Acad Sci,Bach Inst Biochem,Moscow 119071//Russia/; Russian Acad Med Sci,Mechnikov Res Inst Vaccines & Sera,Moscow 103064//Russia/
Journal: APPLIED BIOCHEMISTRY AND MICROBIOLOGY, 2003, V39, N5 (SEP-OCT), P 446-453
ISSN: 0003-6838 Publication date: 20030900
Publisher: MAIK NAUKA/INTERPERIODICA, C/O KLUWER ACADEMIC-PLENUM PUBLISHERS, 233 SPRING ST, NEW YORK, NY 10013-1578 USA
Language: English Document Type: ARTICLE

Geographic Location: Russia
Journal Subject Category: BIOTECHNOLOGY & APPLIED MICROBIOLOGY; MICROBIOLOGY
Abstract: A panel of eight monoclonal antibodies raised against horseradish root peroxidase was assembled and characterized. Affinity constants were determined for all antibodies, and their specificity for various structural forms of the enzyme (native peroxidase, apoperoxidase, and denatured peroxidase) were assessed by competitive enzyme immunoassay. The effects of the antibodies on the process of refolding of peroxidase after its denaturing with 6.5 M guanidine chloride were studied spectrophotometrically, by the restoration of the enzymatic activity in the reaction of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate) oxidation. The yield of the active enzyme in the course of the refolding was increased by 1.5-1.7 times in the presence of antibody H1. Effects of the antibodies constituting the panel on the activity of native peroxidase and the stability of its dilute solutions were analyzed.
Identifiers--KeyWord Plus(R): TRYPTOPHAN FLUORESCENCE; **HORSERADISH - PEROXIDASE** ; MONOCLONAL-ANTIBODIES; ARTIFICIAL **CHAPERONE** ; CARBOXYPEPTIDASE-A; CIRCULAR-DICHROISM; GLYCEROL
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...Identifiers--TRYPTOPHAN FLUORESCENCE; **HORSERADISH - PEROXIDASE** ; MONOCLONAL-ANTIBODIES; ARTIFICIAL **CHAPERONE** ; CARBOXYPEPTIDASE-A; CIRCULAR-DICHROISM; GLYCEROL

6/9,K/19 (Item 5 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

11423825 Genuine Article#: 652CT Number of References: 29

Title: Phospholipid assisted folding of a denatured heme protein: effect of phosphatidylethanolamine

Author(s): Debnath D; Bhattacharya S; Chakrabarti A (REPRINT)
Corporate Source: Saha Inst Nucl Phys,Div Biophys,37 Belgachia Rd/Kolkata 700037//India/ (REPRINT); Saha Inst Nucl Phys,Div Biophys,Kolkata 700037//India/

Journal: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 2003, V301, N4 (FEB 21), P979-984
ISSN: 0006-291X Publication date: 20030221
Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA
Language: English Document Type: ARTICLE
Geographic Location: India
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; BIOPHYSICS

Abstract: The role of the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins have not been done so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its refolding from denatured state. Dimyristoylphosphatidyleth-anolamine (DMPE), a bilayer-forming PE, was able to increase the reactivation yield of denatured HRP upon 30 min refolding at 25degreesC. However, dioleoylphosphatidylethanolamine (DOPE), containing one double bond in the fatty acid chains, which does not favour bilayer organization, did not support proper refolding. The phospholipids with N-methylated head groups, phosphatidylcholines, e.g., DMPC and DOPC showed differential effects when DMPC remained mostly non-supportive while DOPC on the contrary led to inhibition of the refolding of the denatured heme enzyme. Fluorescence spectroscopic studies also indicated changes in the microenvironments of the heme moiety and the single tryptophan residue of HRP in presence of the aminophospholipid. (C) 2003 Elsevier Science (USA). All rights reserved.

Descriptors--Author Keywords: heme protein ; peroxidase ; phosphatidylethanolamine ; fluorescence
Identifiers--KeyWord Plus(R): **HORSERADISH - PEROXIDASE** ; MEMBRANE-PROTEIN; CIRCULAR-DICHROISM; **CHAPERONE**; FLUORESCENCE; STEP

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...Abstract: the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins...

...so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its...
...Identifiers-- **HORSERADISH - PEROXIDASE** ; MEMBRANE-PROTEIN; CIRCULAR-DICHROISM; **CHAPERONE**; FLUORESCENCE; STEP

6/9,K/20 (Item 6 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

11412446 Genuine Article#: 649EZ Number of References: 64
Title: Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone **function**
Author(s): Shin BK; Wang H; Yim AM; Le Naour F; Brichory F; Jang JH; Zhao R ; Puravs E; Tra J; Michael CW; Misek DE (REPRINT) ; Hanash SM
Corporate Source: Univ Michigan,Dept Pediat,1150 W Med Ctr Dr,Rm A520,MSRB-1/Ann Arbor//MI/48109 (REPRINT); Univ Michigan,Dept Pediat,Ann Arbor//MI/48109; Univ Michigan,Dept Pathol,Ann Arbor//MI/48109
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 2003, V278, N9 (FEB 28), P 7607-7616
ISSN: 0021-9258 Publication date: 20030228
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA
Language: English Document Type: ARTICLE
Geographic Location: USA
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: There is currently limited data available pertaining to theglobal characterization of the cell surface proteome. We have implemented a strategy for the comprehensive profiling and identification of surface membrane proteins. This strategy has been applied to cancer cells, including the SH-SY5Y neuroblastoma, the A549 lung adenocarcinoma, the LoVo colon adenocarcinoma, and the Sup-B15 acute lymphoblastic leukemia (B cell) cell lines and ovarian tumor cells. Surface membrane proteins of viable, intact cells were subjected to biotinylation then affinity-captured and purified on monomeric avidin columns. The

biotinylated proteins were eluted from the monomeric avidin columns as intact proteins and were subsequently separated by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained for the different cell populations analyzed. Identification of a subset of biotinylated proteins among the different cell populations analyzed using matrix-assisted laser desorption ionization and tandem mass spectrometry uncovered proteins with a restricted expression pattern in some cell line(s), such as CD87 and the activin receptor type IIB. We also identified more widely expressed proteins, such as CD98, and a sushi repeat-containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70, HSP60, HSP54, HSP27, and protein disulfide isomerase. Comprehensive profiling of the cell surface proteome provides an effective approach for the identification of commonly occurring proteins as well as proteins with restricted expression patterns in this compartment.

Identifiers--KeyWord Plus(R): HEAT-SHOCK PROTEINS; LEADERLESS SECRETORY PATHWAY; PLASMA-MEMBRANE EXPRESSION; FIBROBLAST GROWTH-FACTOR; NATURAL-KILLER-CELLS; HUMAN TUMOR-CELLS; ENDOPLASMIC-RETICULUM; MOLECULAR CHAPERONES; HSP70; IDENTIFICATION

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...**Title: profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with** chaperone **function**
...Abstract: by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained...

...containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70...

6/9,K/21 (Item 7 from file: 34)

11374567 Genuine Article#: 644HU Number of References: 24
Title: Stabilization of heat-induced changes in plant peroxidase preparations by ClpX, a bacterial heat shock protein
Author(s): Kroczynska B (REPRINT) ; Ciesielski A; Sergio L
Corporate Source: Univ Illinois,Dept Med Chem & Pharmacognosy,Mol Biol Res Bldg,900 S Ashland Ave/Chicago//IL/60607 (REPRINT); Polish Acad Sci,Inst Biochem & Biophys,PL-02106 Warsaw//Poland/; Inst Agron

Univ,Inst Orticltura Ind,I-70126 Bari//Italy/
Journal: JOURNAL OF PLANT PHYSIOLOGY, 2002, V159, N12 (DEC), P1295-1299
ISSN: 0176-1617 Publication date: 20021200
Publisher: URBAN & FISCHER VERLAG, BRANCH OFFICE JENA, P O BOX 100537, D-07705 JENA, GERMANY
Language: English Document Type: ARTICLE
Geographic Location: USA; Poland; Italy
Journal Subject Category: PLANT SCIENCES
Abstract: Peroxidases (PODs) are known to be quite stable at elevated temperatures. Moreover, partially denatured peroxidases are able to regain their catalytic activity during incubation at room temperature. In this paper, we describe the effects of some heat shock proteins on the self-reactivation of plant peroxidase preparations. Horseradish and artichoke peroxidases (HRP and ARP, respectively) were first heated (at 60degreesC or 90degreesC), then incubated at a slightly elevated temperature (30degreesC). The heat-treatment resulted in a considerable loss of activity of both enzymes but the subsequent incubation allowed their reactivation. However, no reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding that would result in aggregation.
Descriptors--Author Keywords: ClpX ; **horseradish peroxidase** ; molecular **chaperone** ; protein-protein interaction ; self-renaturation
Identifiers--KeyWord Plus(R): ESCHERICHIA-COLI; **HORSERADISH - PEROXIDASE** ; PURIFICATION; TEMPERATURE; REPLICATION; SUBSTRATE; MECHANISM; STRESS
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...Abstract: reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory

effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding...
...Identifiers--ESCHERICHIA-COLI; **HORSERADISH - PEROXIDASE** ; PURIFICATION; TEMPERATURE; REPLICATION; SUBSTRATE; MECHANISM; STRESS

6/9,K/22 (Item 8 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

10789876 Genuine Article#: 563YR Number of References: 66
Title: S-thiolation of HSP27 regulates its multimeric aggregate size independently of phosphorylation
Author(s): Eaton P (REPRINT) ; Fuller W; Shattock MJ
Corporate Source: St Thomas Hosp,Rayne Inst, Ctr Cardiovasc Biol & Med,London SE1 7EH//England/ (REPRINT); St Thomas Hosp,Rayne Inst, Ctr Cardiovasc Biol & Med,London SE1 7EH//England/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 2002, V277, N24 (JUN 14), P 21189-21196
ISSN: 0021-9258 Publication date: 20020614
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA
Language: English Document Type: ARTICLE
Geographic Location: England
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: HSP27 exists as large aggregates that breakdown after phosphorylation. We show rat cardiac HSP27 is S-thiolated during oxidant stress, and this modification, without phosphorylation, disaggregates multimeric HSP27. Biotinylated cysteine acts as a probe for thiolated proteins, which are detected using non-reducing Western blots probed with streptavidin- **horseradish peroxidase** . Controls show a low level of S-thiolation, which is increased 3.6-fold during post-ischemic reperfusion. S-Thiolated proteins were purified using streptavidin-agarose, and Western immunoblotting showed HSP27 was present. We increased protein S-thiolation 10-fold with 10 mum H2O2 with or without a kinase inhibitor mixture (staurosporine, genistein, bisindolylmaleimide, SB203580, and PD98059). H2O2 alone induced the phosphorylation of HSP27 Ser-86 and Ser-45/Ser-59 of its homologue alphaB crystallin. However, kinase inhibition reduced phosphorylation of these sites below basal. Despite effective kinase inhibition, H2O2 still disaggregated HSP27, but not aB crystallin. This is consistent with the lack of an S-thiolation site on aB crystallin. Thus, we have demonstrated a novel mechanism of HSP27 multimeric size regulation. S-Thiolation must occur at Cys-141, the only cysteine in rat HSP27.
Identifiers--KeyWord Plus(R): HEAT-SHOCK PROTEINS; ALPHA-B-CRYSTALLIN; OXIDATIVE STRESS; CREATINE-KINASE; TROPONIN-I; MOLECULAR **CHAPERONE** ; ENDOTHELIAL-CELLS; CARDIAC MYOCYTES; OXIDANT STRESS; RAT-HEART
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...Abstract: probe for thiolated proteins, which are detected using non-reducing Western blots probed with streptavidin- **horseradish peroxidase** . Controls show a low level of S-thiolation, which is increased 3.6-fold during...
...Identifiers--HEAT-SHOCK PROTEINS; ALPHA-B-CRYSTALLIN; OXIDATIVE STRESS; CREATINE-KINASE; TROPONIN-I; MOLECULAR **CHAPERONE** ; ENDOTHELIAL-CELLS; CARDIAC MYOCYTES; OXIDANT STRESS; RAT-HEART

6/9,K/23 (Item 9 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

10768354 Genuine Article#: 567AQ Number of References: 16
Title: From aggregation to chaperoning: Pressure effect on intermolecular interactions of proteins
Author(s): Smeller L (REPRINT) ; Meersman F; Tolgyesi F; Bode C; Fidy J; Heremans K
Corporate Source: Semmelweis Univ Med,Dept Biophys & Radiat Biol,Puskin U 9 PF 263/H-1444 Budapest//Hungary/ (REPRINT); Semmelweis Univ Med,Dept Biophys & Radiat Biol,H-1444 Budapest//Hungary/; Katholieke Univ Leuven,Dept Chem,B-3001 Louvain//Belgium/
Journal: HIGH PRESSURE RESEARCH, 2002, V22, N3-4,SI (JUN), P751-756
ISSN: 0895-7959 Publication date: 20020600
Publisher: GORDON BREACH PUBLISHING, TAYLOR & FRANCIS GROUP, 325 CHESTNUT ST, 8TH FL, PHILADELPHIA, PA 19106 USA
Language: English Document Type: ARTICLE
Geographic Location: Hungary; Belgium
Journal Subject Category: PHYSICS, MULTIDISCIPLINARY
Abstract: The effect of pressure on the protein aggregation is shown in this paper. Deposition of insoluble protein aggregates is one of the key factors in the conformational diseases. Pressure counteracts the formation of intermolecular beta-structure. Already slight pressurization to typically 2-3kbar can destabilize aggregates of apo-**horseradish peroxidase** . On the other hand, the **chaperone** proteins, which prevent aggregation of damaged proteins exist in big oligomers. We show that pressure treatment of these aggregates changes the **chaperone** activity.
Descriptors--Author Keywords: aggregation ; dissociation ; **chaperone** ; high pressure ; protein ; FTIR ; filbrillogenesis ; amyloid
Identifiers--KeyWord Plus(R): INFRARED-SPECTROSCOPY; ALPHA-CRYSTALLIN; CHYMOTRYPSINOGEN; STABILITY; COLD
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...Abstract: intermolecular beta-structure. Already slight pressurization to typically 2-3kbar can destabilize aggregates of apo- **horseradish peroxidase** . On the other hand, the **chaperone** proteins, which prevent aggregation of damaged proteins exist in big oligomers. We show that pressure treatment of these aggregates changes the **chaperone** activity.

6/9,K/24 (Item 10 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09611643 Genuine Article#: 427YQ Number of References: 38
Title: Binding of a denatured heme protein and ATP to erythroid spectrin
Author(s): Chakrabarti A (REPRINT) ; Bhattacharya S; Ray S; Bhattacharyya M
Corporate Source: Saha Inst Nucl Phys,Div Biophys,37 Belgachia Rd/Calcutta 700037/W Bengal/India/ (REPRINT); Saha Inst Nucl Phys,Div Biophys,Calcutta 700037/W Bengal/India/
Journal: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, 2001, V282, N5 (APR 20), P1189-1193
ISSN: 0006-291X Publication date: 20010420
Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA
Language: English Document Type: ARTICLE
Geographic Location: India
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY; BIOPHYSICS
Abstract: Spectrin is a large, warm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of increasing concentrations of spectrin from that in the absence, This indicated that spectrin could bind denatured HRP and inhibit their refolding. In presence of 1 nM ATP and 10 mg MgCl2 the spectrin binding of denatured HRP is abolished. This activity of decreasing the reactivation yield was found to be ATP-dependent and the denatured enzyme after 30 min refolding in the presence of spectrin, pretreated with Mg/ATP, showed about 40% increase in the reactivation yield compared to the same in absence of spectrin. Fluorescence spectroscopic studies indicated binding of ATP to native spectrin showing concentration-dependent quenching of tryptophan fluorescence by ATP. The apparent dissociation constant of binding of ATP to spectrin was estimated to be 1.1 mM. A high affinity binding of spectrin with denatured HRP has been characterized (K-d = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes, (C) 2001 Academic Press.
Descriptors--Author Keywords: spectrin ; heme protein ; **chaperone** -like activity ; fluorescence ; ATP-binding
Identifiers--KeyWord Plus(R): **HORSERADISH - PEROXIDASE** ; MEMBRANE SKELETON; ALPHA-CRYSTALLIN; FLUORESCENCE; SITES
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...Abstract: warm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of...

...characterized (K-d = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes, (C) 2001 Academic Press.
...Identifiers-- **HORSERADISH - PEROXIDASE** ; MEMBRANE SKELETON; ALPHA-CRYSTALLIN; FLUORESCENCE; SITES

6/9,K/25 (Item 11 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09404386 Genuine Article#: 401HY Number of References: 28
Title: Improvement of productivity of active horseradish peroxidase **in**

Escherichia coli by coexpression of Dsb proteins

Author(s): Kondo A (REPRINT) ; Kohda J; Endo Y; Shiromizu T; Kurokawa Y; Nishihara K; Yanagi H; Yura T; Fukuda H
Corporate Source: Kobe Univ,Fac Engn, Dept Sci & Chem Engn, Nada Ku,1-1 Rokkodai Cho/Kobe/Hyogo 6578501/Japan/ (REPRINT); Kobe Univ,Grad Sch Sci & Technol, Div Mol Sci, Nada Ku,Kobe/Hyogo 6578501/Japan/; Kobe Univ,Fac Engn, Dept Sci & Chem Engn, Nada Ku,Kobe/Hyogo 6578501/Japan/; HSP Res Inst,Kyoto 6008813//Japan/
Journal: JOURNAL OF BIOSCIENCE AND BIOENGINEERING, 2000, V90, N6 (DEC), P 600-606
ISSN: 1389-1723 Publication date: 20001200
Publisher: SOC BIOSCIENCE BIOENGINEERING JAPAN, OSAKA UNIV, FACULTY ENGINEERING, 2-1 YAMADAOKA, SUITA, OSAKA, 565-0871, JAPAN
Language: English Document Type: ARTICLE
Geographic Location: Japan
Journal Subject Category: BIOTECHNOLOGY & APPLIED MICROBIOLOGY; FOOD SCIENCE & TECHNOLOGY
Abstract: Coexpression of two classes of folding accessory proteins, molecular chaperones and foldases, can be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in Escherichia coli. The effects of coexpression of a series of folding accessory proteins (DnaK, DnaJ, GrpE, GroEL/ES, trigger factor (TF), DsbA, DsbB, DsbC, DsbD, and thioredoxin (Trx)) on the productivity of active HRP in E, coli were examined. Active HRP was produced by very mild induction with 1 muM isopropyl-beta -D-thiogalactopyranoside (IPTG) at 37 degreesC, whereas the amount of active HRP produced by the induction with 1 mM IPTG was negligibly small. Active HRP production was increased significantly by coexpression of DsbA-DsbB (DsbAB) or DsbC-DsbD (DsbCD), while coexpression of molecular chaperones did not improve active HRP production. The growth of E. coli cells was inhibited significantly by the induction with 1 mM IPTG in a HRP single expression system. In contrast, when HRP was coexpressed with DsbCD, the growth inhibition of E. coli was not observed. Therefore, coexpression of Dsb proteins improves both the cell growth and the productivity of HRP.
Descriptors--Author Keywords: molecular **chaperone** ; foldase ; coexpression ; protein folding ; Escherichia coli
Identifiers--KeyWord Plus(R): DISULFIDE-ISOMERASE; TRIGGER FACTOR; GROEL-GROES; EXPRESSION; DNAK; PERIPLASM; GENE; POLYPEPTIDE; CHAPERONES; BONDS
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Title: Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**
...Abstract: be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in...

6/9,K/26 (Item 12 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08055633 Genuine Article#: 241TX Number of References: 21
Title: Spectrin exhibits chaperone **-like activity**
Author(s): Chakrabarti A (REPRINT) ; Bhattacharya S
Corporate Source: SAHA INST NUCL PHYS,DIV BIOPHYS, 37 BELGACHIA RD/CALCUTTA 700037/W BENGAL/INDIA/ (REPRINT)
Journal: CURRENT SCIENCE, 1999, V77, N6 (SEP 25), P812-813
ISSN: 0011-3891 Publication date: 19990925
Publisher: CURRENT SCIENCE ASSN, C V RAMAN AVENUE, PO BOX 8005, BANGALORE 560 080, INDIA
Language: English Document Type: ARTICLE
Geographic Location: INDIA
Subfile: CC PHYS--Current Contents, Physical, Chemical & Earth Sciences; CC AGRI--Current Contents, Agriculture, Biology & Environmental Sciences
Journal Subject Category: MULTIDISCIPLINARY SCIENCES
Abstract: Erythroid spectrin, a cytoskeletal protein, inhibits refolding of denatured heme enzyme, **horseradish peroxidase** (HRP). The denatured enzyme after 30 min refolding in the absence and presence of spectrin showed significant differences in the enzyme activity. The enzymic activity of HRP decreased in the presence of spectrin when compared with the activity in absence of spectrin. This inhibitory effect of spectrin is abolished when it was preincubated with magnesium-ATP indicating that spectrin exhibits **chaperone** -like activity.
Identifiers--KeyWord Plus(R): MOLECULAR **CHAPERONE** ; ALPHA-CRYSTALLIN; FLUORESCENCE; PROTEINS; BINDING; MEMBRANES
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Title: Spectrin exhibits chaperone **-like activity**
Abstract: Erythroid spectrin, a cytoskeletal protein, inhibits refolding of denatured heme enzyme, **horseradish peroxidase** (HRP). The denatured enzyme after 30 min refolding in the absence and presence of spectrin ...

...of spectrin is abolished when it was preincubated with magnesium-ATP indicating that spectrin exhibits **chaperone** -like activity.
...Identifiers--MOLECULAR **CHAPERONE** ; ALPHA-CRYSTALLIN; FLUORESCENCE; PROTEINS; BINDING; MEMBRANES

6/9,K/27 (Item 13 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

07785628 Genuine Article#: 208DE Number of References: 40
Title: Functional early endosomes are required for maturation of major histocompatibility complex class II molecules in human B lymphoblastoid cells
Author(s): Pond L; Watts C (REPRINT)
Corporate Source: UNIV DUNDEE,DEPT BIOCHEM, WELLCOME TRUST BLDG/DUNDEE DD1 4HN//SCOTLAND/ (REPRINT); UNIV DUNDEE,DEPT BIOCHEM/DUNDEE DD1 4HN//SCOTLAND/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1999, V274, N25 (JUN 18), P 18049-18054
ISSN: 0021-9258 Publication date: 19990618
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814
Language: English Document Type: ARTICLE
Geographic Location: SCOTLAND
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must be degraded to enable peptide capture by MHC class II molecules. It remains controversial exactly which route or routes MHC class II/Ii complexes take to reach the sites of Ii processing and peptide loading. We have asked whether early endosomes

are required for successful maturation of MHC class II molecules by using an in situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas transport of secretory Ig through the secretory pathway is virtually normal in the ablated cells, newly synthesized MHC class II/Ii complexes never reach compartments capable of processing Ii. These results strongly suggest that the transport of the bulk of newly synthesized MHC class II molecules through early endosomes is obligatory and that direct input into later endosomes/lysosomes does not take place.

Identifiers--KeyWord Plus(R): MHC-CLASS-II; INVARIANT CHAIN COMPLEXES; ENDOCYTIC PATHWAY; SUBCELLULAR COMPARTMENTS; LYSOSOMAL COMPARTMENTS; INTRACELLULAR PATHWAY; PEPTIDE COMPLEXES; CYTOPLASMIC TAIL; GOLGI-COMPLEX; HLA ANTIGENS

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Abstract: Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must...
...situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin-**horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas...

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DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06449239 Genuine Article#: YU212 Number of References: 32
Title: Detection of glycated fetal human lens crystallins by Concanavalin-A and aldehyde staining
Author(s): Ahrend MHJ; Bours J (REPRINT)
Corporate Source: UNIV BONN,INST EXPT OPHTHALMOL, SIGMUND FREUD STR 25/D-53105 BONN//GERMANY/ (REPRINT); UNIV BONN,INST EXPT OPHTHALMOL/D-53105 BONN//GERMANY/
Journal: MECHANISMS OF AGEING AND DEVELOPMENT, 1997, V99, N3 (DEC 30), P 167-179
ISSN: 0047-6374 Publication date: 19971230
Publisher: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND
Language: English Document Type: ARTICLE
Geographic Location: GERMANY
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: GERIATRICS & GERONTOLOGY; BIOCHEMISTRY & MOLECULAR BIOLOGY; CELL BIOLOGY
Abstract: The binding of the lectin Concanavalin-A (Con-A) to crystallins was investigated. For this purpose, human fetal and juvenile lens crystallins were isofocused and specifically stained brown for glycoproteins by the lectin Con-A, and purple by the periodic acid Schiff's reagent (PAS). In reference experiments protein bands were stained with Coomassie Blue for proteins. Since Con-A is a protein with glucose-specific receptors, the following crystallins, glycated with this sugar, were visualized after isoelectric focusing: HM-, beta(L),-, beta(S),- and gamma-crystallins, but not alpha(L),- and beta(H),-crystallins. Glycation increased significantly with fetal age. The crystallins themselves could also function as sugar receptors, because it was shown that they possessed also receptors for glucose, like Con-A. This crystallin receptor staining revealed beta(L),-, beta(S),-, gamma- but hardly HM-crystallins. The PAS, Con-A and receptor stainings gave in principle identical results. The glycoproteins Con-A, **horseradish peroxidase** and lentil lectins were used as positive controls. (C) 1997 Elsevier Science Ireland Ltd.
Descriptors--Author Keywords: glycation ; isoelectric focusing ; lectin staining ; periodic acid Schiff aldehyde staining ; development ; human fetal lens ; HM-, alpha-, beta-, beta(s)-, gamma-crystallins
Identifiers--KeyWord Plus(R): AGE-RELATED-CHANGES; ADULT HUMAN LENS; MOLECULAR **CHAPERONE** ; ALPHA-CRYSTALLINS; PROTEINS; WATER; GLYCOPROTEINS; GLYCOSYLATION; INVIVO
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...Abstract: PAS, Con-A and receptor stainings gave in principle identical results. The glycoproteins Con-A, **horseradish peroxidase** and lentil lectins were used as positive controls. (C) 1997 Elsevier Science Ireland Ltd.
...Identifiers--AGE-RELATED-CHANGES; ADULT HUMAN LENS; MOLECULAR **CHAPERONE**; ALPHA-CRYSTALLINS; PROTEINS; WATER; GLYCOPROTEINS; GLYCOSYLATION; INVIVO

6/9,K/29 (Item 15 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06001276 Genuine Article#: XN360 Number of References: 91
Title: Constitutive expression of the 27-kDa heat shock protein (Hsp27) in sensory and motor neurons of the rat nervous system
Author(s): Plumier JCL (REPRINT) ; Hopkins DA; Robertson HA; Currie RW
Corporate Source: DALHOUSIE UNIV,DEPT ANAT & NEUROBIOL, FAC MED/HALIFAX/NS B3H 4H7/CANADA/ (REPRINT); DALHOUSIE UNIV,DEPT PHARMACOL, FAC MED/HALIFAX/NS B3H 4H7/CANADA/
Journal: JOURNAL OF COMPARATIVE NEUROLOGY, 1997, V384, N3 (AUG 4), P409-428
ISSN: 0021-9967 Publication date: 19970804
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012
Language: English Document Type: ARTICLE

Geographic Location: CANADA
Subfile: CC LIFE--Current Contents, Life Sciences
Journal Subject Category: NEUROSCIENCES
Abstract: In this study, the constitutive expression of the 27-kDa heat shock protein (Hsp27) in the adult rat central nervous system has been examined by immunohistochemistry and by two-dimensional gel Western blot analysis. Hsp27 immunoreactivity was observed primarily in motoneurons of cranial nerve nuclei and spinal cord, and in primary sensory neurons and their central processes. Also, Hsp27 immunoreactivity was present in neurons of the arcuate nucleus and of the reticular formation. However, only a subset of these neurons was Hsp27-immunoreactive. Most general somatic efferent motoneurons of the hypoglossal nucleus and spinal motor columns and most special visceral efferent motoneurons of the cranial nerve nuclei were Hsp27-positive. In contrast, fewer general somatic efferent motoneurons for eye muscles were Hsp27-positive, and only a small proportion of general visceral efferent neurons, i.e., parasympathetic and sympathetic preganglionic neurons, were stained for Hsp27. Many pseudounipolar sensory neurons were Hsp27-immunoreactive, and the patterns of staining in central sensory nuclei suggested that specific subpopulations of sensory neurons contained Hsp27. The cellular distribution of Hsp27 was uniform throughout the cytoplasm, including the perikaryon, axon and dendrites, the latter often exhibiting varicosities or beading in distal processes. Western blot analyses revealed that at least three phosphorylated isoforms of Hsp27 were present in the spinal cord. These results suggest that constitutively expressed Hsp27 may be related to functional subpopulations of motoneurons and primary sensory neurons.

Descriptors--Author Keywords: arcuate nucleus ; cranial nerve nuclei ; brainstem ; spinal cord ; mesencephalic trigeminal nucleus
Identifiers--KeyWord Plus(R): ALPHA-B-CRYSTALLIN; UPPER ALIMENTARY-TRACT; TRIGEMINAL PRIMARY AFFERENTS; STEM RETICULOSPINAL NUCLEI; **HORSERADISH - PEROXIDASE** ; MESSENGER-RNA; ACTIN POLYMERIZATION; HYPOGLOSSAL NUCLEUS; IMMUNOHISTOCHEMICAL LOCALIZATION; VISCEROTOPIC REPRESENTATION
Research Fronts: 95-2215 001 (CHAPERONIN GROEL; PROTEIN TRANSLOCATION; HSP70 OF SACCHAROMYCES-CEREVISIAE; ATPASE CYCLE; ASYMMETRICAL INTERACTION)
95-3190 001 (INCREASED ABUNDANCE OF SPECIFIC SKELETAL-MUSCLE PROTEIN-TYROSINE PHOSPHATASES; ALPHA-B-CRYSTALLIN EXPRESSION)
95-6910 001 (MOUSE ALPHA-B-CRYSTALLIN SMALL HEAT-SHOCK PROTEIN GENE; IN-VITRO MOLECULAR **CHAPERONE** ACTIVITY; RABBIT LENS CELLS)
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...Identifiers--ALPHA-B-CRYSTALLIN; UPPER ALIMENTARY-TRACT; TRIGEMINAL PRIMARY AFFERENTS; STEM RETICULOSPINAL NUCLEI; **HORSERADISH - PEROXIDASE** ; MESSENGER-RNA; ACTIN POLYMERIZATION; HYPOGLOSSAL NUCLEUS; IMMUNOHISTOCHEMICAL LOCALIZATION; VISCEROTOPIC REPRESENTATION
...Research Fronts: 95-6910 001 (MOUSE ALPHA-B-CRYSTALLIN SMALL HEAT-SHOCK PROTEIN GENE; IN-VITRO MOLECULAR **CHAPERONE** ACTIVITY; RABBIT LENS CELLS)

6/9,K/30 (Item 16 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02277760 Genuine Article#: KP884 Number of References: 51
Title: ORGANELLE-SPECIFIC PHOSPHORYLATION - IDENTIFICATION OF UNIQUE MEMBRANE PHOSPHOPROTEINS OF THE ENDOPLASMIC-RETICULUM AND ENDOSOMAL APPARATUS
Author(s): RINDRESS D; LEI X; AHLUWALIA JPS; CAMERON PH; FAZEL A; POSNER BI ; BERGERON JJM
Corporate Source: MCGILL UNIV,DEPT ANAT & CELL BIOL/MONTREAL H3A 2B2/QUEBEC/CANADA/; MCGILL UNIV,DEPT MED/MONTREAL H3A 2B2/QUEBEC/CANADA/
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1993, V268, N7 (MAR 5), P 5139-5147
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Geographic Location: CANADA
Subfile: SciSearch; CC LIFE--Current Contents, Life Sciences
Journal Subject Category: BIOCHEMISTRY & MOLECULAR BIOLOGY
Abstract: Highly purified endoplasmic reticulum fractions from rat liver and dog pancreas harbor membrane-associated kinases that phosphorylate integral membrane proteins of 90, 56, 35, and 15 kDa with [gamma-P-32] GTP and of 90, 56, and 35 kDa with [gamma-P32]ATP. Of these, only the 35-kDa phosphoprotein was N-glycosylated. Screening of Golgi fractions, endosomes, plasma membranes, lysosomes, and mitochondria revealed phosphoproteins unique to each organelle. In particular, endosomes were found to harbor a 48-kDa extrinsic membrane protein and two or more

integral membrane phosphoproteins of 30-35 kDa. None of these were N-glycosylated as judged by their insensitivity to digestion by N-glycosidase F and a lack of binding to concanavalin A or wheat germ agglutinin. Since the 30-35-kDa membrane phosphoproteins present in Golgi-free endosomal fractions were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique to endosomes. Since membrane phosphoproteins unique to the endoplasmic reticulum have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P. H., Ou, W.-J., Doherty, J.-J., II, Louvard, D., Bell, A. W., Dignard, D., Thomas, D. Y., and Bergeron, J. J. M. (1991) J. Biol. Chem. 266, 19599-19610; Ahluwalia, N., Bergeron, J. J. M., Wada, I., Degen, E., and Williams, D. B. (1992) J. Biol. Chem. 267, 10914-10918), then the unique endosomal phosphoproteins may serve equally important functions in addition to serving as novel markers for the organelle.

Identifiers--KeyWords Plus: RAT-LIVER; GOLGI FRACTIONS; PRELYSOSOMAL COMPARTMENT; LYSOSOMAL MEMBRANE; BINDING-PROTEINS; RECEPTOR KINASE; INSULIN; ANTIBODIES; INVIVO; CELLS

Research Fronts: 91-3106 001 (IDENTIFICATION OF A 40X10(3)MR CENTROMERE-ASSOCIATED PROTEIN; ACTIN ISOFORM EXPRESSION IN CULTURED ARTERIAL SMOOTH-MUSCLE CELLS)

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WILSON JM, 1987, V105, P691, J CELL BIOL

...Abstract: were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique...

...have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P. H., Ou, W.-J., Doherty, J.-J., II...

6/9,K/31 (Item 1 from file: 71)

03276305 2003056345

Stabilization of heat-induced changes in plant peroxidase preparations by ClpX, a bacterial heat shock protein

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EMAIL: kroczyb@uic.edu

Journal: Journal of Plant Physiology, 159/12 (1295-1299), 2002, Germany
PUBLICATION DATE: December 1, 2002
CODEN: JPPHE
ISSN: 0176-1617
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 24

Peroxidases (PODs) are known to be quite stable at elevated temperatures. Moreover, partially denatured peroxidases are able to regain their catalytic activity during incubation at room temperature. In this paper, we describe the effects of some heat shock proteins on the self-reactivation of plant peroxidase preparations. Horseradish and artichoke peroxidases (HRP and ARP, respectively) were first heated (at 60degreesC or 90degreesC), then incubated at a slightly elevated temperature

(30degreesC). The heat-treatment resulted in a considerable loss of activity of both enzymes but the subsequent incubation allowed their reactivation. However, no reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding that would result in aggregation.

DESCRIPTORS:
ClpX; **Horseradish peroxidase** ; Molecular **chaperone** ; Protein-protein interaction; Self-renaturation

SPECIES DESCRIPTORS:
Armoracia rusticana; Bacteria (microorganisms); Cynara scolymus

CLASSIFICATION CODE AND DESCRIPTION:
92.1.4.2 - PLANT SCIENCE / BIOCHEMISTRY / Enzymes / Activity, kinetics
92.1.1.6 - PLANT SCIENCE / BIOCHEMISTRY / Molecular Biology / Proteins
82.2.8 - PROTEIN BIOCHEMISTRY / STRUCTURAL STUDIES / Folding, Unfolding and Stability
82.6.2 - PROTEIN BIOCHEMISTRY / OXIDOREDUCTIVE ENZYMES (EC 1.) / Antioxidant Enzymes
82.12.10 - PROTEIN BIOCHEMISTRY / OTHER PROTEINS / Heat Shock Proteins

...reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding...

DESCRIPTORS:
ClpX; **Horseradish peroxidase** ; Molecular **chaperone** ; Protein-protein interaction; Self-renaturation

6/9,K/32 (Item 2 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

03226227 2006040194
Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking
Zeng X.; Overmeyer J.H.; Maltese W.A.
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EMAIL: wmaltese@meduohio.edu
Journal: Journal of Cell Science, 119/2 (259-270), 2006, United Kingdom
PUBLICATION DATE: January 15, 2006
CODEN: JNCSA
ISSN: 0021-9533
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 86

Beclin 1 was originally identified as a novel Bcl-2-interacting protein, but co-immunoprecipitation studies suggest that the major physiological partner for Beclin 1 is the mammalian class III phosphatidylinositol 3-kinase (PI 3-kinase) Vps34. Beclin 1 has been proposed to function as a tumor suppressor by promoting cellular macroautophagy, a process that is known to depend on Vps34. However, an alternative role for Beclin 1 in modulating normal Vps34-dependent protein trafficking pathways has not been ruled out. This possibility was examined in U-251 glioblastoma cells. Immunoprecipitates of endogenous Beclin 1 contained human Vps34 (hVps34), but not Bcl-2. Suppression of Beclin 1 expression by short interfering (si)RNA-mediated gene silencing blunted the autophagic response of the cells to nutrient deprivation or CSUB2-ceramide. However, other PI 3-kinase-dependent trafficking pathways, such as the post-endocytic sorting of the epidermal growth factor receptor (EGFR) or the proteolytic processing of procathepsin D en route from the trans-Golgi network (TGN) to lysosomes, were not affected. Depletion of Beclin 1 did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin 1 functions mainly to engage hVps34 in the autophagic pathway.

DESCRIPTORS:
Beclin; Autophagy; Vps34; Phosphatidylinositol-3-kinase; Cell death; Endocytosis; Glioblastoma
SPECIES DESCRIPTORS:
Mammalia; Armoracia rusticana

CLASSIFICATION CODE AND DESCRIPTION:
89.2.5.1 - CELL AND DEVELOPMENTAL BIOLOGY / CELL GROWTH AND DIVISION / Cellular Senescence and Death / Autophagy
89.1.7.1 - CELL AND DEVELOPMENTAL BIOLOGY / MEMBRANES AND CELL TRANSPORT / Endocytosis, Exocytosis and Intracellular Transport / Endocytosis
89.1.8.4 - CELL AND DEVELOPMENTAL BIOLOGY / MEMBRANES AND CELL TRANSPORT / Cytoplasmic Membranes / Lysosomes and peroxisomes

MOLECULAR SEQUENCE DATABANK NUMBER:
GENBANK/AF077301/(REFERRED NUMBER)

...affected. Depletion of Beclin 1 did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function ...

...hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin...

6/9,K/33 (Item 3 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

03193141 2006010633
Development and optimization of a useful assay for determining Hsp90's

inherent ATPase activity

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EMAIL: bblagg@ku.edu
Journal: Bioorganic and Medicinal Chemistry, 14/4 (1134-1142), 2006, United Kingdom
PUBLICATION DATE: February 15, 2006
CODEN: BMECE
ISSN: 0968-0896
PUBLISHER ITEM IDENTIFIER: S0968089605008886
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 43

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced. (c) 2005 Elsevier Ltd. All rights reserved.

DESCRIPTORS:
Hsp90; Inhibitors; Assay development; Cancer

SPECIES DESCRIPTORS:
Armoracia rusticana; Animalia

CLASSIFICATION CODE AND DESCRIPTION:
99 - General

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...
...optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0
...

6/9,K/34 (Item 4 from file: 71)

02384283 2003167388

Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone **function**

Shin B.K.; Wang H.; Yim A.M.; Le Naour F.; Brichory F.; Jang J.H.; Zhao R.; Puravs E.; Tra J.; Michael C.W.; Misek D.E.; Hanash S.M.
ADDRESS: D.E. Misek, Dept. of Pediatrics, University of Michigan, 1150 West Medical Center Dr., Ann Arbor, MI 48109-0656, United States

EMAIL: dmisek@umich.edu
Journal: Journal of Biological Chemistry, 278/9 (7607-7616), 2003, United States
PUBLICATION DATE: February 28, 2003
CODEN: JBCHA
ISSN: 0021-9258
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 64

There is currently limited data available pertaining to the global characterization of the cell surface proteome. We have implemented a strategy for the comprehensive profiling and identification of surface membrane proteins. This strategy has been applied to cancer cells, including the SH-SY5Y neuroblastoma, the A549 lung adenocarcinoma, the LoVo colon adenocarcinoma, and the Sup-B15 acute lymphoblastic leukemia (B cell) cell lines and ovarian tumor cells. Surface membrane proteins of viable, intact cells were subjected to biotinylation then affinity-captured and purified on monomeric avidin columns. The biotinylated proteins were eluted from the monomeric avidin columns as intact proteins and were subsequently separated by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained for the different cell populations analyzed. Identification of a subset of biotinylated proteins among the different cell populations analyzed using matrix-assisted laser desorption ionization and tandem mass spectrometry uncovered proteins with a restricted expression pattern in some cell line(s), such as CD87 and the activin receptor type IIB. We also identified more widely expressed proteins, such as CD98, and a sushi repeat-containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70, HSP60, HSP54, HSP27, and protein disulfide isomerase. Comprehensive profiling of the cell surface proteome provides an effective approach for the identification of commonly occurring proteins as well as proteins with restricted expression patterns in this compartment.

CLASSIFICATION CODE AND DESCRIPTION:
82.2.8 - PROTEIN BIOCHEMISTRY / STRUCTURAL STUDIES / Folding, Unfolding and Stability
82.3.2 - PROTEIN BIOCHEMISTRY / PROTEIN ENGINEERING / Peptide/Protein Libraries

...**profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with** chaperone **function**

...by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained...

...containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70...

6/9,K/35 (Item 5 from file: 71)

02278574 2003062720

Phospholipid assisted folding of a denatured heme protein: Effect of phosphatidylethanolamine

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Journal: Biochemical and Biophysical Research Communications, 301/4 (979-984), 2003, United States
PUBLICATION DATE: February 21, 2003
CODEN: BBRCA
ISSN: 0006-291X
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 29

The role of the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins have not been done so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its refolding from denatured state. Dimyristoylphosphatidylethanolamine (DMPE), a bilayer-forming PE, was able to increase the reactivation yield of denatured HRP upon 30min refolding at 25degreesC. However, dioleoylphosphatidylethanolamine (DOPE), containing one double bond in the fatty acid chains, which does not favour bilayer organization, did not support proper refolding. The phospholipids with N-methylated head groups, phosphatidylcholines, e.g., DMPC and DOPC showed differential effects when DMPC remained mostly non-supportive while DOPC on the contrary led to inhibition of the refolding of the denatured heme enzyme. Fluorescence spectroscopic studies also indicated changes in the microenvironments of the heme moiety and the single tryptophan residue of HRP in presence of the aminophospholipid.

DESCRIPTORS:
Heme protein; Peroxidase; Phosphatidylethanolamine; Fluorescence

CLASSIFICATION CODE AND DESCRIPTION:
82.2.8 - PROTEIN BIOCHEMISTRY / STRUCTURAL STUDIES / Folding, Unfolding and Stability

...the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins...

...so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its...

6/9,K/36 (Item 6 from file: 71)

01859991 2001221597

Binding of a denatured heme protein and ATP to erythroid spectrin

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Journal: Biochemical and Biophysical Research Communications, 282/5 (1189-1193), 2001, United States
CODEN: BBRCA
ISSN: 0006-291X
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 38

Spectrin is a large, worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of increasing concentrations of spectrin from that in the absence. This indicated that spectrin could bind denatured HRP and inhibit their refolding. In presence of 1 mM ATP and 10 mM MgClSUB2 the spectrin binding of denatured HRP is abolished. This activity of decreasing the reactivation yield was found to be ATP-dependent and the denatured enzyme after 30 min refolding in the presence of spectrin, pretreated with Mg/ATP, showed about 40% increase in the reactivation yield compared to the same in absence of spectrin. Fluorescence spectroscopic studies indicated binding of ATP to native spectrin showing concentration-dependent quenching of tryptophan fluorescence by ATP. The apparent dissociation constant of binding of ATP to spectrin was estimated to be 1.1 mM. A high affinity binding of spectrin with denatured HRP has been characterized (KSUBd = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes. (c) 2001 Academic Press.

DESCRIPTORS:
Spectrin; Heme protein; **Chaperone** -like activity; Fluorescence; ATP-binding

CLASSIFICATION CODE AND DESCRIPTION:
92.1.4.1 - PLANT SCIENCE / BIOCHEMISTRY / Enzymes / Purification and structure
92.1.1.6 - PLANT SCIENCE / BIOCHEMISTRY / Molecular Biology / Proteins

...worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of...

...been characterized (KSUBd = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes. (c) 2001 Academic Press.

DESCRIPTORS:
Spectrin; Heme protein; **Chaperone** -like activity; Fluorescence;

ATP-binding

6/9,K/37 (Item 7 from file: 71)

01646691 2001019654
Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**
Kondo A.; Kohda J.; Endo Y.; Shiromizu T.; Kurokawa Y.; Nishihara K.; Yanagi H.; Yura T.; Fukuda H.
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Journal: Journal of Bioscience and Bioengineering, 90/6 (600-606), 2000, Japan
CODEN: JBBIF
ISSN: 1389-1723
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 28

Coexpression of two classes of folding accessory proteins, molecular chaperones and foldases, can be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in Escherichia coli. The effects of coexpression of a series of folding accessory proteins (DnaK, DnaJ, GrpE, GroEL/ES, trigger factor (TF), DsbA, DsbB, DsbC, DsbD, and thioredoxin (Trx)) on the productivity of active HRP in E. coli were examined. Active HRP was produced by very mild induction with 1 muM isopropyl-beta-D-thiogalactopyranoside (IPTG) at 37degreesC, whereas the amount of active HRP produced by the induction with 1 mM IPTG was negligibly small. Active HRP production was increased significantly by coexpression of DsbA-DsbB (DsbAB) or DsbC-DsbD (DsbCD), while coexpression of molecular chaperones did not improve active HRP production. The growth of E. coli cells was inhibited significantly by the induction with 1 mM IPTG in a HRP single expression system. In contrast, when HRP was coexpressed with DsbCD, the growth inhibition of E. coli was not observed. Therefore, coexpression of Dsb proteins improves both the cell growth and the productivity of HRP.

DESCRIPTORS:
Molecular **chaperone** ; Foldase; Coexpression; Protein folding; Escherichia coli

SPECIES DESCRIPTORS:
Escherichia coli
CLASSIFICATION CODE AND DESCRIPTION:
85.1.3.2 - APPLIED MICROBIOLOGY AND BIOTECHNOLOGY / BIOTECHNOLOGY - TECHNIQUES AND PROCEDURES / Culture Selection and Improvement / Recombinant DNA technology
85.7.2 - APPLIED MICROBIOLOGY AND BIOTECHNOLOGY / MICROBIAL METABOLISM AND PHYSIOLOGY / Microbial Energetics
82.2.8 - PROTEIN BIOCHEMISTRY / STRUCTURAL STUDIES / Folding, Unfolding and Stability

82.6.2.2 - PROTEIN BIOCHEMISTRY / OXIDOREDUCTIVE ENZYMES (EC 1.) / Antioxidant Enzymes / Glutathione peroxidase
82.12.7.3 - PROTEIN BIOCHEMISTRY / OTHER PROTEINS / Microbial Proteins / Bacterial

Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**

...be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in...

DESCRIPTORS:
Molecular **chaperone** ; Foldase; Coexpression; Protein folding; Escherichia coli

6/9,K/38 (Item 8 from file: 71)

01170704 1999139680
Functional early endosomes are required for maturation of major histocompatibility complex class II molecules in human B lymphoblastoid cells
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EMAIL: c.watts@dundee.ac.uk
Journal: Journal of Biological Chemistry, 274/25 (18049-18054), 1999, United States
PUBLICATION DATE: June 18, 1999
CODEN: JBCHA
ISSN: 0021-9258
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English
NO. OF REFERENCES: 41

Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must be degraded to enable peptide capture by MHC class II molecules. It remains controversial exactly which route or routes MHC class II/Ii complexes take to reach the sites of Ii processing and peptide loading. We have asked whether early endosomes are required for successful maturation of MHC class II molecules by using an in situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas transport of secretory Ig through the secretory pathway is virtually normal in the ablated cells, newly synthesized MHC class II/Ii complexes never reach compartments capable of processing Ii. These results strongly suggest that the transport of the bulk of newly synthesized MHC class II molecules through early endosomes is obligatory and that direct input into later endosomes/lysosomes does not take place.

CLASSIFICATION CODE AND DESCRIPTION:
86.6.1.1 - IMMUNOLOGY AND INFECTIOUS DISEASES / TRANSPLANTATION IMMUNOLOGY / Major Histocompatibility Antigens / Biochemistry
89.1.1.3 - CELL AND DEVELOPMENTAL BIOLOGY / MEMBRANES AND CELL TRANSPORT / Cell Surface and Plasma Membrane / Proteins and glycoproteins
89.1.7.1 - CELL AND DEVELOPMENTAL BIOLOGY / MEMBRANES AND CELL TRANSPORT / Endocytosis, Exocytosis and Intracellular Transport / Endocytosis
Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must...

...situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin-**horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas...

6/9,K/39 (Item 1 from file: 73)

14217358 EMBASE No: 2006614747

Expression and prognostic significance of prothymosin-alpha and ERp57 in human gastric cancer

Leys C.M.; Nomura S.; LaFleur B.J.; Ferrone S.; Kaminishi M.; Montgomery E.; Goldenring J.R.
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Surgery (SURGERY) (United States) 2007, 141/1 (41-50)
CODEN: SURGA ISSN: 0039-6060
PUBLISHER ITEM IDENTIFIER: S0039606006003461
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 31

Purpose: Prothymosin-alpha and ERp57 were previously identified as markers for gastric metaplasia in a mouse model of Helicobacter-induced gastric metaplasia and neoplasia. In this paper we assess whether the expression of these putative biomarkers in humans is correlated with gastric metaplasia and adenocarcinoma and clinical outcomes. Methods: Eight tissue microarrays, containing 749 paraffin-embedded tissue cores from 164 gastric cancer patients, were stained for prothymosin-alpha and ERp57 by **horseradish peroxidase** immunohistochemical techniques. The proportion of stained cells per core was quantitated using the Ariol SL-50 automated image analysis system. Results: Prothymosin-alpha stained a significantly higher percentage of nuclei in cancer and metastases compared with normal gastric mucosa. ERp57 staining was significantly decreased in cancer and metastases compared with both normal gastric mucosa and metaplasias. ERp57 expression also correlated with greater depth of tumor invasion and advanced stage of disease. Kaplan-Meier survival analysis determined that tumors with the highest quartile of ERp57 expression were statistically associated with longer postoperative survival. A Cox proportional hazard analysis showed that maintenance of ERp57 expression was associated with longer postoperative survival. Conclusions: These results suggest that although prothymosin-alpha is overexpressed in gastric adenocarcinoma, it

is not associated with alterations in survival. In contrast, loss of ERp57 expression correlated with more aggressive disease and could provide useful prognostic information for gastric cancer patients. (c) 2007 Mosby, Inc. All rights reserved.

DRUG DESCRIPTORS:
* **chaperone** --endogenous compound--ec; *major histocompatibility antigen class 1--endogenous compound--ec; *prothymosin alpha--endogenous compound --ec; *trefoil peptide--endogenous compound--ec
monoclonal antibody; polypeptide--endogenous compound--ec
MEDICAL DESCRIPTORS:
*intestine metaplasia; *stomach cancer--etiology--et; *stomach cancer --surgery--su
Kaplan Meier method; article; cancer survival; female; human tissue; human; immunohistochemistry; major clinical study; male; priority journal; prognosis; protein expression; stomach adenocarcinoma--etiology--et; stomach adenocarcinoma--surgery--su; stomach carcinogenesis
DRUG TERMS (UNCONTROLLED): ERp57 protein
CAS REGISTRY NO.: 89964-14-7 (prothymosin alpha)
SECTION HEADINGS:
  016 Cancer
  026 Immunology, Serology and Transplantation
  048 Gastroenterology
  005 General Pathology and Pathological Anatomy
  009 Surgery

  ...tissue cores from 164 gastric cancer patients, were stained for prothymosin-alpha and ERp57 by **horseradish peroxidase** immunohistochemical techniques. The proportion of stained cells per core was quantitated using the Ariol SL...
DRUG DESCRIPTORS:
* **chaperone** --endogenous compound--ec; *major histocompatibility antigen class 1--endogenous compound--ec; *prothymosin alpha--endogenous compound ...

6/9,K/40 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
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13588188 EMBASE No: 2006073322
Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking
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W.A. Maltese, Department of Biochemistry and Cancer Biology, Medical University of Ohio, Toledo, OH 43614 United States
AUTHOR EMAIL: wmaltese@meduohio.edu
Journal of Cell Science (J. CELL SCI.) (United Kingdom) 15 JAN 2006, 119/2 (259-270)
CODEN: JNCSA ISSN: 0021-9533
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 86

Beclin 1 was originally identified as a novel Bcl-2-interacting protein, but co-immunoprecipitation studies suggest that the major physiological partner for Beclin 1 is the mammalian class III phosphatidylinositol

3-kinase (PI 3-kinase) Vps34. Beclin 1 has been proposed to function as a tumor suppressor by promoting cellular macroautophagy, a process that is known to depend on Vps34. However, an alternative role for Beclin 1 in modulating normal Vps34-dependent protein trafficking pathways has not been ruled out. This possibility was examined in U-251 glioblastoma cells. Immunoprecipitates of endogenous Beclin 1 contained human Vps34 (hVps34), but not Bcl-2. Suppression of Beclin 1 expression by short interfering (si)RNA-mediated gene silencing blunted the autophagic response of the cells to nutrient deprivation or CSUB2-ceramide. However, other PI 3-kinase-dependent trafficking pathways, such as the post-endocytic sorting of the epidermal growth factor receptor (EGFR) or the proteolytic processing of procathepsin D en route from the trans-Golgi network (TGN) to lysosomes, were not affected. Depletion of Beclin 1 did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin 1 functions mainly to engage hVps34 in the autophagic pathway.
;
MOLECULAR SEQUENCE NUMBER: GENBANK, AF077301
DRUG DESCRIPTORS:
*lysosome enzyme--endogenous compound--ec; *phosphatidylinositol 3 kinase --endogenous compound--ec
small interfering RNA--endogenous compound--ec; ceramide--endogenous compound--ec; epidermal growth factor receptor--endogenous compound--ec; cathepsin D--endogenous compound--ec; **horseradish peroxidase** --endogenous compound--ec; **chaperone** --endogenous compound--ec; adaptor protein--endogenous compound--ec; unclassified drug
MEDICAL DESCRIPTORS:
*enzyme specificity; *autophagy; *endocytosis
immunoprecipitation; cancer inhibition; enzyme activity; enzyme localization; cell degeneration; protein transport; glioblastoma; cancer cell; protein expression; gene expression; protein content; enzyme inhibition; gene silencing; protein targeting; protein degradation; Golgi complex; lysosome; cell transport; protein depletion; cell compartmentalization; cell swelling; human; controlled study; human cell; article; nucleotide sequence; priority journal
DRUG TERMS (UNCONTROLLED): protein beclin 1--endogenous compound--ec
CAS REGISTRY NO.: 115926-52-8 (phosphatidylinositol 3 kinase); 9025-26-7 (cathepsin D)
SECTION HEADINGS:
005 General Pathology and Pathological Anatomy
008 Neurology and Neurosurgery
016 Cancer

...affected. Depletion of Beclin 1 did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function...

...hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin...
DRUG DESCRIPTORS:
...endogenous compound--ec; epidermal growth factor receptor--endogenous

compound--ec; cathepsin D--endogenous compound--ec; **horseradish peroxidase** --endogenous compound--ec; **chaperone** --endogenous compound--ec; adaptor protein--endogenous compound--ec; unclassified drug

6/9,K/41 (Item 3 from file: 73)

13537456 EMBASE No: 2006020047

Development and optimization of a useful assay for determining Hsp90's inherent ATPase activity

Avila C.; Kornilayev B.A.; Blagg B.S.J.
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CODEN: BMECE ISSN: 0968-0896
PUBLISHER ITEM IDENTIFIER: S0968089605008886
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 43

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced. (c) 2005 Elsevier Ltd. All rights reserved.

DRUG DESCRIPTORS:
*heat shock protein 90--endogenous compound--ec
adenosine triphosphatase; phosphorylase; glucose oxidase; **horseradish peroxidase** ; geldanamycin--drug analysis--an; geldanamycin--drug development--dv; geldanamycin--pharmacology--pd; radicicol--drug analysis --an; radicicol--drug development--dv; radicicol--pharmacology--pd; adenosine triphosphate
MEDICAL DESCRIPTORS:
*enzyme assay
enzyme activity; enzyme inhibition; protein folding; conformational transition; protein denaturation; cancer; signal transduction; protein degradation; cancer chemotherapy; enzyme binding; nonhuman; animal cell; article
CAS REGISTRY NO.: 37289-25-1, 9000-83-3 (adenosine triphosphatase); 9035-74-9 (phosphorylase); 9001-37-0 (glucose oxidase); 30562-34-6 (geldanamycin); 12772-57-5 (radicicol); 15237-44-2, 56-65-5, 987-65-5 (adenosine triphosphate)
SECTION HEADINGS:
029 Clinical and Experimental Biochemistry
030 Clinical and Experimental Pharmacology

037 Drug Literature Index

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...

...optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0 ...
DRUG DESCRIPTORS:
adenosine triphosphatase; phosphorylase; glucose oxidase; **horseradish peroxidase** ; geldanamycin--drug analysis--an; geldanamycin--drug development--dv; geldanamycin--pharmacology--pd; radicicol--drug analysis --an...

6/9,K/42 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE

12165529 EMBASE No: 2003269650
Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone **function**
Shin B.K.; Wang H.; Yim A.M.; Le Naour F.; Brichory F.; Jang J.H.; Zhao R.; Puravs E.; Tra J.; Michael C.W.; Misek D.E.; Hanash S.M.
D.E. Misek, Dept. of Pediatrics, University of Michigan, 1150 West Medical Center Dr., Ann Arbor, MI 48109-0656 United States
AUTHOR EMAIL: dmisek@umich.edu
Journal of Biological Chemistry (J. BIOL. CHEM.) (United States) 28 FEB 2003, 278/9 (7607-7616)
CODEN: JBCHA ISSN: 0021-9258
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 64

There is currently limited data available pertaining to the global characterization of the cell surface proteome. We have implemented a strategy for the comprehensive profiling and identification of surface membrane proteins. This strategy has been applied to cancer cells, including the SH-SY5Y neuroblastoma, the A549 lung adenocarcinoma, the LoVo colon adenocarcinoma, and the Sup-B15 acute lymphoblastic leukemia (B cell) cell lines and ovarian tumor cells. Surface membrane proteins of viable, intact cells were subjected to biotinylation then affinity-captured and purified on monomeric avidin columns. The biotinylated proteins were eluted from the monomeric avidin columns as intact proteins and were subsequently separated by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained for the different cell populations analyzed. Identification of a subset of biotinylated proteins among the different cell populations analyzed using matrix-assisted laser desorption ionization and tandem mass spectrometry uncovered proteins with a restricted expression pattern in some cell line(s), such as CD87 and the activin receptor type IIB. We also identified more widely expressed proteins, such as CD98, and a sushi repeat-containing protein, a member of the selectin family. Remarkably, a set of proteins

identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70, HSP60, HSP54, HSP27, and protein disulfide isomerase. Comprehensive profiling of the cell surface proteome provides an effective approach for the identification of commonly occurring proteins as well as proteins with restricted expression patterns in this compartment.
DRUG DESCRIPTORS:
*cell surface protein--endogenous compound--ec; * **chaperone** --endogenous compound--ec
avidin; polyvinylidene fluoride; streptavidin; **horseradish peroxidase** ; activin receptor 2--endogenous compound--ec; CD98 antigen--endogenous compound--ec; protein; selectin; protein disulfide isomerase; heat shock protein 70; heat shock protein 60; heat shock protein 27; membrane protein --endogenous compound--ec; unclassified drug
MEDICAL DESCRIPTORS:
*protein analysis; *neuroblastoma; *lung adenocarcinoma; *colon adenocarcinoma; *acute lymphoblastic leukemia; *ovary tumor
cancer cell culture; protein function; cell viability; biotinylation; protein purification; elution; polyacrylamide gel electrophoresis; molecular hybridization; reproducibility; cell population; matrix assisted laser desorption ionization time of flight mass spectrometry; protein expression; protein localization; human; controlled study; human cell; article; priority journal
DRUG TERMS (UNCONTROLLED): CD87 antigen; protein GRP78; protein grp75; heat shock protein 54
CAS REGISTRY NO.: 24937-79-9 (polyvinylidene fluoride); 9013-20-1 (streptavidin); 67254-75-5 (protein); 37318-49-3 (protein disulfide isomerase)
SECTION HEADINGS:
016 Cancer
029 Clinical and Experimental Biochemistry

...profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone **function**

...by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained...

...containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70...
DRUG DESCRIPTORS:
*cell surface protein--endogenous compound--ec; * **chaperone** --endogenous compound--ec
avidin; polyvinylidene fluoride; streptavidin; **horseradish peroxidase** ; activin receptor 2--endogenous compound--ec; CD98 antigen--endogenous compound--ec; protein; selectin; protein disulfide...

6/9,K/43 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE

11989618 EMBASE No: 2003100447
Phospholipid assisted folding of a denatured heme protein: Effect of

phosphatidylethanolamine

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AUTHOR EMAIL: abhijit@biop.saha.ernet.in
Biochemical and Biophysical Research Communications (BIOCHEM. BIOPHYS. RES. COMMUN.) (United States) 21 FEB 2003, 301/4 (979-984)
CODEN: BBRCA ISSN: 0006-291X
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 29

The role of the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins have not been done so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its refolding from denatured state. Dimyristoylphosphatidylethanolamine (DMPE), a bilayer-forming PE, was able to increase the reactivation yield of denatured HRP upon 30min refolding at 25degreesC. However, dioleoylphosphatidylethanolamine (DOPE), containing one double bond in the fatty acid chains, which does not favour bilayer organization, did not support proper refolding. The phospholipids with N-methylated head groups, phosphatidylcholines, e.g., DMPC and DOPC showed differential effects when DMPC remained mostly non-supportive while DOPC on the contrary led to inhibition of the refolding of the denatured heme enzyme. Fluorescence spectroscopic studies also indicated changes in the microenvironments of the heme moiety and the single tryptophan residue of HRP in presence of the aminophospholipid.

DRUG DESCRIPTORS:
*phosphatidylethanolamine; *dimyristoylphosphatidylcholine; *dioleoylphosphatidylcholine; *dioleoylphosphatidylethanolamine; *hemoprotein
phospholipid; **horseradish peroxidase** ; fatty acid; tryptophan; unclassified drug
MEDICAL DESCRIPTORS:
protein folding; protein denaturation; chemical bond; bilayer membrane; methylation; fluorescence spectroscopy; microenvironment; enzyme activity; energy transfer; enzyme inhibition; article; priority journal
DRUG TERMS (UNCONTROLLED): dimyristoylphosphatidylethanolamine
CAS REGISTRY NO.: 1405-71-6 (phosphatidylethanolamine); 13699-48-4, 18194-24-6 (dimyristoylphosphatidylcholine); 10015-85-7 (dioleoylphosphatidylcholine); 2462-63-7 (dioleoylphosphatidylethanolamine); 20255-95-2 (dimyristoylphosphatidylethanolamine); 6912-86-3, 73-22-3 (tryptophan)
SECTION HEADINGS:
029 Clinical and Experimental Biochemistry

...the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins...

...so far and in particular with the heme proteins. We have used the heme

enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its...
DRUG DESCRIPTORS:
phospholipid; **horseradish peroxidase** ; fatty acid; tryptophan; unclassified drug

6/9,K/44 (Item 6 from file: 73)

11968770 EMBASE No: 2003078524
Hsp70 is present in human saliva
Fabian T.K.; Gaspar J.; Fejerdy L.; Kaan B.; Balint M.; Csermely P.; Fejerdy P.
Prof. P. Csermely, Department of Medical Chemistry, Semmelweis University, P. O. Box 260, H-1444 Budapest Hungary
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Medical Science Monitor (MED. SCI. MONIT.) (Poland) 01 JAN 2003, 9/1 (BR62-BR65)
CODEN: MSMOF ISSN: 1234-1010
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 14

Background: There is increasing evidence that chaperones are also present outside the cell, exerting cytokine-like effects and influencing immune recognition. Hsp70 has been found to be present in human blood sera. Chaperonins Cpn10 and Cpn60 are present in pancreatic juice, but Hsp70 is not. These observations raise the possibility that molecular chaperones may be present in other secretory fluids, such as human saliva.
Material/Methods: Human whole saliva was collected from six participants under resting conditions and secretory stimulation. The samples were precleared by centrifugation and sterile filtered. Salivary volume, protein concentration and amylase activity were determined. For detection of Hsp70 saliva proteins were separated on a 12.5% SDS-PAGE. Semi-dry Western blot analysis was used with a primary antibody against the inducible form of Hsp70. Hsp70 bands were detected with a **horseradish peroxidase** -linked secondary antibody and ECL-Western blotting analysis. Results: A single band was recognized around 70 kDa in the saliva of all the participants. There was a significant decrease of Hsp70, and a non-significant decrease of total protein concentration during stimulation, whereas the activity of salivary amylase increased significantly. Stimulation significantly increased the Hsp70, total protein and amylase outputs as well as the amylase/protein ratio, and decreased the Hsp70/amylase and Hsp70/protein ratios. Conclusions: Hsp70 is secreted to saliva, but unlike amylase is not transported by the exocytotic secretory mechanisms of acinar cells. Passive transport mechanisms of Hsp70 from blood serum or from salivary gland cells may be major routes of salivary Hsp70 secretion.

DRUG DESCRIPTORS:
*heat shock protein 70
amylase; **chaperone**
MEDICAL DESCRIPTORS:
*saliva analysis
protein analysis; acinar cell; protein transport; protein secretion; salivation; centrifugation; enzyme activity; polyacrylamide gel

electrophoresis; signal transduction; Western blotting; passive transport; human; human experiment; normal human; controlled study; article
CAS REGISTRY NO.: 9000-90-2, 9000-92-4, 9001-19-8 (amylase)
SECTION HEADINGS:
  011 Otorhinolaryngology
  029 Clinical and Experimental Biochemistry

  ...a primary antibody against the inducible form of Hsp70. Hsp70 bands were detected with a **horseradish peroxidase** -linked secondary antibody and ECL-Western blotting analysis. Results: A single band was recognized around...
DRUG DESCRIPTORS:
amylase; **chaperone**

6/9,K/45 (Item 7 from file: 73)
DIALOG(R)File 73:EMBASE

11331247 EMBASE No: 2001345533
Binding of a denatured heme protein and ATP to erythroid spectrin
Chakrabarti A.; Bhattacharya S.; Ray S.; Bhattacharyya M.
A. Chakrabarti, Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta 700037 India
AUTHOR EMAIL: abhijit@biop.saha.ernet.in
Biochemical and Biophysical Research Communications (BIOCHEM. BIOPHYS. RES. COMMUN.) (United States) 2001, 282/5 (1189-1193)
CODEN: BBRCA ISSN: 0006-291X
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 38

Spectrin is a large, worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of increasing concentrations of spectrin from that in the absence. This indicated that spectrin could bind denatured HRP and inhibit their refolding. In presence of 1 mM ATP and 10 mM MgClSUB2 the spectrin binding of denatured HRP is abolished. This activity of decreasing the reactivation yield was found to be ATP-dependent and the denatured enzyme after 30 min refolding in the presence of spectrin, pretreated with Mg/ATP, showed about 40% increase in the reactivation yield compared to the same in absence of spectrin. Fluorescence spectroscopic studies indicated binding of ATP to native spectrin showing concentration-dependent quenching of tryptophan fluorescence by ATP. The apparent dissociation constant of binding of ATP to spectrin was estimated to be 1.1 mM. A high affinity binding of spectrin with denatured HRP has been characterized (KSUBd = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes. (c) 2001 Academic Press.

DRUG DESCRIPTORS:
*hemoprotein--endogenous compound--ec; *adenosine triphosphate--endogenous compound--ec; *spectrin--endogenous compound--ec
cytoskeleton protein--endogenous compound--ec; **horseradish peroxidase** --endogenous compound--ec; magnesium chloride; tryptophan; **chaperone**
MEDICAL DESCRIPTORS:

*protein binding; *protein denaturation
protein folding; fluorescence spectroscopy; concentration response; dissociation constant; binding affinity; erythrocyte; cell type; mathematical analysis; goat; nonhuman; animal cell; article; priority journal
CAS REGISTRY NO.: 15237-44-2, 56-65-5, 987-65-5 (adenosine triphosphate); 12634-43-4 (spectrin); 7786-30-3, 7791-18-6 (magnesium chloride); 6912-86-3, 73-22-3 (tryptophan)
SECTION HEADINGS:
025 Hematology
029 Clinical and Experimental Biochemistry

...worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of...

...been characterized (KSUBd = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes. (c) 2001 Academic Press.
DRUG DESCRIPTORS:
cytoskeleton protein--endogenous compound--ec; **horseradish peroxidase** --endogenous compound--ec; magnesium chloride; tryptophan; **chaperone**

6/9,K/46 (Item 8 from file: 73)
DIALOG(R)File 73:EMBASE

10989333 EMBASE No: 2001033613
Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**
Kondo A.; Kohda J.; Endo Y.; Shiromizu T.; Kurokawa Y.; Nishihara K.; Yanagi H.; Yura T.; Fukuda H.
A. Kondo, Dept. of Chemical Science/Eng., Faculty of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501 Japan
Journal of Bioscience and Bioengineering (J. BIOSCI. BIOENG.) (Japan) 2000, 90/6 (600-606)
CODEN: JBBIF ISSN: 1389-1723
DOCUMENT TYPE: Journal ; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 28

Coexpression of two classes of folding accessory proteins, molecular chaperones and foldases, can be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in Escherichia coli. The effects of coexpression of a series of folding accessory proteins (DnaK, DnaJ, GrpE, GroEL/ES, trigger factor (TF), DsbA, DsbB, DsbC, DsbD, and thioredoxin (Trx)) on the productivity of active HRP in E. coli were examined. Active HRP was produced by very mild induction with 1 muM isopropyl-beta-D-thiogalactopyranoside (IPTG) at 37degreesC, whereas the amount of active HRP produced by the induction with 1 mM IPTG was negligibly small. Active HRP production was increased significantly by coexpression of DsbA-DsbB (DsbAB) or DsbC-DsbD (DsbCD), while coexpression

of molecular chaperones did not improve active HRP production. The growth of E. coli cells was inhibited significantly by the induction with 1 mM IPTG in a HRP single expression system. In contrast, when HRP was coexpressed with DsbCD, the growth inhibition of E. coli was not observed. Therefore, coexpression of Dsb proteins improves both the cell growth and the productivity of HRP.

DRUG DESCRIPTORS:
* **horseradish peroxidase** ; *protein
chaperone ; recombinant protein; thioredoxin; bacterial enzyme--endogenous compound--ec; pyranoside
MEDICAL DESCRIPTORS:
*Escherichia coli
protein folding; disulfide bond; enzyme synthesis; cell growth; growth inhibition; protein expression; protein synthesis; enzyme activity; nonhuman; controlled study; article
CAS REGISTRY NO.: 67254-75-5 (protein); 52500-60-4 (thioredoxin)
SECTION HEADINGS:
004 Microbiology: Bacteriology, Mycology, Parasitology and Virology

Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**

...be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in...
DRUG DESCRIPTORS:
* **horseradish peroxidase** ; *protein
chaperone ; recombinant protein; thioredoxin; bacterial enzyme--endogenous compound--ec; pyranoside

6/9,K/47 (Item 9 from file: 73)

07731867 EMBASE No: 1999214136
Functional early endosomes are required for maturation of major histocompatibility complex class II molecules in human B lymphoblastoid cells
Pond L.; Watts C.
C. Watts, Department of Biochemistry, Wellcome Trust Building, University of Dundee, Dundee DD1 4HN United Kingdom
AUTHOR EMAIL: c.watts@dundee.ac.uk
Journal of Biological Chemistry (J. BIOL. CHEM.) (United States) 18 JUN 1999, 274/25 (18049-18054)
CODEN: JBCHA ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 41

Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must be degraded to enable peptide capture by MHC class II molecules. It remains controversial exactly which route or routes MHC class II/Ii

complexes take to reach the sites of Ii processing and peptide loading. We have asked whether early endosomes are required for successful maturation of MHC class II molecules by using an in situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas transport of secretory Ig through the secretory pathway is virtually normal in the ablated cells, newly synthesized MHC class II/Ii complexes never reach compartments capable of processing Ii. These results strongly suggest that the transport of the bulk of newly synthesized MHC class II molecules through early endosomes is obligatory and that direct input into later endosomes/lysosomes does not take place.

DRUG DESCRIPTORS:
*HLA antigen class 2--endogenous compound--ec
chaperone --endogenous compound--ec; **horseradish peroxidase** ; diaminobenzidine; transferrin; secretory immunoglobulin--endogenous compound--ec
MEDICAL DESCRIPTORS:
*protein processing; *endosome
pre b lymphocyte; cell compartmentalization; molecular dynamics; protein targeting; lysosome; protein determination; transport kinetics; human; controlled study; human cell; article; priority journal
CAS REGISTRY NO.: 7411-49-6, 91-95-2 (diaminobenzidine); 82030-93-1 (transferrin)
SECTION HEADINGS:
026 Immunology, Serology and Transplantation
029 Clinical and Experimental Biochemistry

Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must...

...situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas...
DRUG DESCRIPTORS:
chaperone --endogenous compound--ec; **horseradish peroxidase** ; diaminobenzidine; transferrin; secretory immunoglobulin--endogenous compound--ec

6/9,K/48 (Item 10 from file: 73)

05313556 EMBASE No: 1993081641
Organelle-specific phosphorylation. Identification of unique membrane phosphoproteins of the endoplasmic reticulum and endosomal apparatus
Rindress D.; Lei X.; Ahluwalia J.P.S.; Cameron P.H.; Fazel A.; Posner B.I.; Bergeron J.J.M.
Department of Anatomy/Cell Biology, McGill University,Montreal, Que. H3A 2B2 Canada
Journal of Biological Chemistry (J. BIOL. CHEM.) (United States) 1993, 268/7 (5139-5147)

CODEN: JBCHA ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Highly purified endoplasmic reticulum fractions from rat liver and dog pancreas harbor membrane-associated kinases that phosphorylate integral membrane proteins of 90, 56, 35, and 15 kDa with (gamma-sup 3sup 2P) GTP and of 90, 56, and 35 kDa with (gamma-sup 3sup 2P)ATP. Of these, only the 35-kDa phosphoprotein was N-glycosylated. Screening of Golgi fractions, endosomes, plasma membranes, lysosomes, and mitochondria revealed phosphoproteins unique to each organelle. In particular, endosomes were found to harbor a 48-kDa extrinsic membrane protein and two or more integral membrane phosphoproteins of 30-35 kDa. None of these were N-glycosylated as judged by their insensitivity to digestion by N-glycosidase F and a lack of binding to concanavalin A or wheat germ agglutinin. Since the 30-35-kDa membrane phosphoproteins present in Golgi-free endosomal fractions were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique to endosomes. Since membrane phosphoproteins unique to the endoplasmic reticulum have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P. H., Ou, W.-J., Doherty, J.-J., II, Louvard, D., Bell, A. W., Dignard, D., Thomas, D. Y., and Bergeron, J. J. M. (1991) J. Biol. Chem. 266, 19599-19610; Ahluwalia, N., Bergeron, J. J. M., Wada, I., Degen, E., and Williams, D. B. (1992) J. Biol. Chem. 267, 10914-10918), then the unique endosomal phosphoproteins may serve equally important functions in addition to serving as novel markers for the organelle.

DRUG DESCRIPTORS:
*membrane protein--endogenous compound--ec; *phosphoprotein--endogenous compound--ec
biochemical marker
MEDICAL DESCRIPTORS:
*endoplasmic reticulum; *endosome; *phosphorylation
animal cell; animal tissue; article; calcium binding; cell membrane; dog; golgi complex; liver; lysosome; mitochondrion; nonhuman; pancreas; priority journal; rat
SECTION HEADINGS:
029 Clinical and Experimental Biochemistry

...were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique...

...have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P. H., Ou, W.-J., Doherty, J.-J., II...

6/9,K/49 (Item 1 from file: 144)

17732668 PASCAL No.: 06-0326406

Development and optimization of a useful assay for determining Hsp90's inherent ATPase activity

AVILA Christopher; KORNILAYEV Boris A; BLAGG Brian S J

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Document Type: P (Serial) ; A (Analytic)

Country of Publication: United Kingdom

Language: English

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced.

English Descriptors: Optimization; Adenosinetriphosphatase; Biological activity; **Chaperone** ; Conformation; Polypeptide; Inhibition; Antineoplastic agent; Signal transduction; Maltose phosphorylase; Glucose oxidase; Peroxidase; Inhibitor; Tumor; Enzymatic activity; Test method; Heat shock protein; Peptides

Broad Descriptors: Hydrolases; Enzyme; Hexosyltransferases; Glycosyltransferases; Transferases; Oxidoreductases; Peroxidases; Hydrolases; Enzyme; Hexosyltransferases; Glycosyltransferases; Transferases; Oxidoreductases; Peroxidases; Hydrolases; Enzima; Hexosyltransferases; Glycosyltransferases; Transferases; Oxidoreductases; Peroxidases

French Descriptors: Optimisation; Adenosinetriphosphatase; Activite biologique; Chaperon; Conformation; Polypeptide; Inhibition; Anticancereux; Transduction signal; Maltose phosphorylase; Glucose oxidase; Peroxidase; Inhibiteur; Tumeur; Activite enzymatique; Methode essai; Proteine choc thermique; Peptide; Proteine Hsp90

Classification Codes: 002B02R01

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...

... optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0 ...

English Descriptors: Optimization; Adenosinetriphosphatase; Biological activity; **Chaperone** ; Conformation; Polypeptide; Inhibition; Antineoplastic agent; Signal transduction; Maltose phosphorylase; Glucose oxidase; Peroxidase; Inhibitor; Tumor; Enzymatic...

6/9,K/50 (Item 2 from file: 144)

16026600 PASCAL No.: 03-0173723

Stabilization of heat-induced changes in plant peroxidase preparations by ClpX, a bacterial heat shock protein

KROCZYNSKA Barbara; CIESIELSKI Arkadiusz; SERGIO Lucrezia
Institute of Biochemistry and Biophysics Polish Academy of Sciences, ul. Pawinskiego 5a, 02-106 Warsaw, Poland; Instituto sull'Orticoltura Industriale do Instituto di Agronomia dell'Universita, via G. Amendola n. 165/A, 70126 Bari, Italy

Journal: Journal of plant physiology, 2002, 159 (12) 1295-1299
ISSN: 0176-1617 CODEN: JPPHEY Availability: INIST-922; 354000107492090020
No. of Refs.: 24 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Germany
Language: English

Peroxidases (PODs) are known to be quite stable at elevated temperatures. Moreover, partially denatured peroxidases are able to regain their catalytic activity during incubation at room temperature. In this paper, we describe the effects of some heat shock proteins on the self-reactivation of plant peroxidase preparations. Horseradish and artichoke peroxidases (HRP and ARP, respectively) were first heated (at 60 Degree C or 90 Degree C), then incubated at a slightly elevated temperature (30 Degree C). The heat-treatment resulted in a considerable loss of activity of both enzymes but the subsequent incubation allowed their reactivation. However, no reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding that would result in aggregation.

English Descriptors: Peroxidase; Thermal stability; Heat shock protein; Pretreatment; High temperature; Enzymatic activity; **Chaperone** ; Regulation(control); Denaturation renaturation; Cynara scolymus; Protein; Molecular interaction
Broad Descriptors: Peroxidases; Oxidoreductases; Enzyme; Compositae; Dicotyledones; Angiospermae; Spermatophyta; Peroxidases; Oxidoreductases; Enzyme; Compositae; Dicotyledones; Angiospermae; Spermatophyta; Peroxidases; Oxidoreductases; Enzima; Compositae; Dicotyledones; Angiospermae; Spermatophyta

French Descriptors: Peroxidase; Stabilite thermique; Proteine choc thermique; Pretraitement; Haute temperature; Activite enzymatique; Chaperon; Regulation; Denaturation renaturation; Cynara scolymus; Proteine; Interaction moleculaire

Classification Codes: 002A10E05; 002A02E02

... reactivation could be detected when incubation was carried out in the presence of the molecular **chaperone** ClpX. Other chaperones that were tested (DnaK, DnaJ and GrpE) did not show the inhibitory effect. Electrophoretic analyses further indicated that the heat-treated **horseradish peroxidase** , but not the native enzyme, binds to ClpX eliminating the possibility of undesirable protein refolding...

English Descriptors: Peroxidase; Thermal stability; Heat shock protein; Pretreatment; High temperature; Enzymatic activity; **Chaperone** ; Regulation(control); Denaturation renaturation; Cynara scolymus; Protein; Molecular interaction

6/9,K/51 (Item 3 from file: 144)

14965851 PASCAL No.: 01-0118910

Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**

KONDO Akihiko; KOHDA Jiro; ENDO Yasunori; SHIROMIZU Tokuhisa; KUROKAWA Yoichi; NISHIHARA Kazuyo; YANAGI Hideki; YURA Takashi; FUKUDA Hideki

Department of Chemical Science and Engineering, Faculty of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan; Division of Molecular Science, Graduate School of Science and Technology, SUP 2 Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan; HSP Research Institute, Kyoto Research Park, Kyoto 600-8813, Japan

Journal: Journal of bioscience and bioengineering, 2000, 90 (6) 600-606
ISSN: 1389-1723 Availability: INIST-8234; 354000094103590030
No. of Refs.: 28 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: Japan
Language: English

Coexpression of two classes of folding accessory proteins, molecular chaperones and foldases, can be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in Escherichia coli. The effects of coexpression of a series of folding accessory proteins (DnaK, DnaJ, GrpE, GroEL/ES, trigger factor (TF), DsbA, DsbB, DsbC, DsbD, and thioredoxin (Trx)) on the productivity of active HRP in E. coli were examined. Active HRP was produced by very mild induction with 1 mu M isopropyl- beta -D-thiogalactopyranoside (IPTG) at 37 Degree C, whereas the amount of active HRP produced by the induction with 1 mM IPTG was negligibly small. Active HRP production was increased significantly by coexpression of DsbA-DsbB (DsbAB) or DsbC-DsbD (DsbCD), while coexpression of molecular chaperones did not improve active HRP production. The growth of E. coli cells was inhibited significantly by the induction with 1 mM IPTG in a HRP single expression system. In contrast, when HRP was coexpressed with DsbCD, the growth inhibition of E. coli was not observed. Therefore, coexpression of Dsb proteins improves both the cell growth and the productivity of HRP.

English Descriptors: Production; Escherichia coli; Horse radish; Peroxidase ; Refolding; **Chaperone** ; Gene; Microorganism culture; Recombinant protein; Gene expression; Gene coexpression
Broad Descriptors: Enterobacteriaceae; Bacteria; Peroxidases; Oxidoreductases; Enzyme; Enterobacteriaceae; Bacterie; Peroxidases; Oxidoreductases; Enzyme; Enterobacteriaceae; Bacteria; Peroxidases; Oxidoreductases; Enzima
French Descriptors: Production; Escherichia coli; Raifort; Peroxidase; Repliement; Chaperon; Gene; Culture microorganisme; Proteine recombinante ; Expression genique; Coexpression genique

Classification Codes: 002A31C02A8; 215

Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb proteins**
... be expected to improve the productivity of soluble and active recombinant proteins. In this study, **horseradish peroxidase** (HRP), which has four disulfide bonds, was selected as a model enzyme and overexpressed in...

English Descriptors: Production; Escherichia coli; Horse radish; Peroxidase ; Refolding; **Chaperone** ; Gene; Microorganism culture; Recombinant protein; Gene expression; Gene coexpression

6/9,K/52 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

20681293 PMID: 16390869
Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking.
Zeng Xuehuo; Overmeyer Jean H; Maltese William A
Department of Biochemistry and Cancer Biology, Medical University of Ohio, Toledo, OH 43614, USA.
Journal of cell science (England) Jan 15 2006, 119 (Pt 2) p259-70, ISSN 0021-9533--Print Journal Code: 0052457
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Beclin 1 was originally identified as a novel Bcl-2-interacting protein, but co-immunoprecipitation studies suggest that the major physiological partner for Beclin 1 is the mammalian class III phosphatidylinositol 3-kinase (PI 3-kinase) Vps34. Beclin 1 has been proposed to function as a tumor suppressor by promoting cellular macroautophagy, a process that is known to depend on Vps34. However, an alternative role for Beclin 1 in modulating normal Vps34-dependent protein trafficking pathways has not been ruled out. This possibility was examined in U-251 glioblastoma cells. Immunoprecipitates of endogenous Beclin 1 contained human Vps34 (hVps34), but not Bcl-2. Suppression of Beclin 1 expression by short interfering

(si)RNA-mediated gene silencing blunted the autophagic response of the cells to nutrient deprivation or C2-ceramide. However, other PI 3-kinase-dependent trafficking pathways, such as the post-endocytic sorting of the epidermal growth factor receptor (EGFR) or the proteolytic processing of procathepsin D en route from the trans-Golgi network (TGN) to lysosomes, were not affected. Depletion of Beclin 1 did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function of hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin 1 functions mainly to engage hVps34 in the autophagic pathway.

Descriptors: *1-Phosphatidylinositol 3-Kinase--metabolism--ME; *Autophagy --physiology--PH; *Endocytosis--physiology--PH; *Lysosomes--enzymology--EN; *Proteins--metabolism--ME; *Transport Vesicles--metabolism--ME; 1-Phosphatidylinositol 3-Kinase--genetics--GE; Animals; Cell Line, Tumor; Humans; Microtubule-Associated Proteins--genetics--GE; Microtubule-Associated Proteins--metabolism--ME; Multiprotein Complexes; Protein Transport --physiology--PH; Proteins--genetics--GE; Proto-Oncogene Proteins c-bcl-2 --genetics--GE; Proto-Oncogene Proteins c-bcl-2--metabolism--ME; RNA, Small Interfering--genetics--GE; RNA, Small Interfering--metabolism--ME; Recombinant Fusion Proteins--genetics--GE; Recombinant Fusion Proteins --metabolism--ME; Research Support, U.S. Gov't, Non-P.H.S.; Signal Transduction--physiology--PH; trans-Golgi Network--metabolism--ME

CAS Registry No.: 0 (BECN1 protein, human); 0 (Microtubule-Associated Proteins); 0 (Multiprotein Complexes); 0 (Proteins); 0 (Proto-Oncogene Proteins c-bcl-2); 0 (RNA, Small Interfering); 0 (Recombinant Fusion Proteins); 0 (light chain 3, human)

Enzyme No.: EC 2.7.1.137 (1-Phosphatidylinositol 3-Kinase)

Record Date Created: 20060113

Record Date Completed: 20060509

Date of Electronic Publication: 20060103

... affected. Depletion of Beclin 1 did not reduce endocytic internalization of a fluid phase marker (**horseradish peroxidase** , HRP) or cause swelling of late endosomal compartments typically seen in cells where the function...

... hVps34 is impaired. These findings argue against a role for Beclin 1 as an essential **chaperone** or adaptor for hVps34 in normal vesicular trafficking, and they support the hypothesis that Beclin...

6/9,K/53 (Item 2 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

20240283 PMID: 15745946

The multiple functions of cysteine-string protein analyzed at Drosophila nerve terminals.

Bronk Peter; Nie Zhiping; Klose Markus K; Dawson-Scully Ken; Zhang Jinhui ; Robertson R Meldrum; Atwood Harold L; Zinsmaier Konrad E

Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, Arizona 85721-0077, USA.

Journal of neuroscience - the official journal of the Society for Neuroscience (United States) Mar 2 2005, 25 (9) p2204-14, ISSN

1529-2401--Electronic Journal Code: 8102140
Contract/Grant No.: R01NS038274; NS; NINDS
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

The synaptic vesicle-associated cysteine-string protein (CSP) is important for synaptic transmission. Previous studies revealed multiple defects at neuromuscular junctions (NMJs) of csp null-mutant Drosophila, but whether these defects are independent of each other or mechanistically linked through J domain mediated-interactions with heat-shock cognate protein 70 (Hsc70) has not been established. To resolve this issue, we genetically dissected the individual functions of CSP by an in vivo structure/function analysis. Expression of mutant CSP lacking the J domain at csp null-mutant NMJs fully restored normal thermo-tolerance of evoked transmitter release but did not completely restore evoked release at room temperature and failed to reverse the abnormal intraterminal Ca2+ levels. This suggests that J domain-mediated functions are essential for the regulation of intraterminal Ca2+ levels but only partially required for regulating evoked release and not required for protecting evoked release against thermal stress. Hence, CSP can also act as an Hsc70-independent **chaperone** protecting evoked release from thermal stress. Expression of mutant CSP lacking the L domain restored neurotransmission and partially reversed the abnormal intraterminal Ca2+ levels, suggesting that the L domain is important, although not essential, for the role of CSP in regulating intraterminal Ca2+ levels. We detected no effects of csp mutations on individual presynaptic Ca2+ signals triggered by action potentials, suggesting that presynaptic Ca2+ entry is not primarily impaired. Both the J and L domains were also required for the role of CSP in synaptic growth. Together, these results suggest that CSP has several independent synaptic functions, affecting synaptic growth, evoked release, thermal protection of evoked release, and intraterminal Ca2+ levels at rest and during stimulation.

Descriptors: *HSP40 Heat-Shock Proteins--physiology--PH; *Membrane Proteins--physiology--PH; *Neuromuscular Junction--cytology--CY; *Point Mutation--physiology--PH; *Presynaptic Terminals--metabolism--ME; Analysis of Variance; Animals; Animals, Genetically Modified; Calcium--metabolism --ME; Calcium Signaling--physiology--PH; Comparative Study; Diagnostic Imaging--methods--MT; Drosophila; Drosophila Proteins--metabolism--ME; Evolution; Gene Expression--genetics--GE; HSP40 Heat-Shock Proteins --chemistry--CH; HSP40 Heat-Shock Proteins--genetics--GE; **Horseradish Peroxidase** --metabolism--ME; Humans; Immunohistochemistry--methods--MT; Membrane Potentials--physiology--PH; Membrane Proteins--chemistry--CH; Membrane Proteins--genetics--GE; Neuromuscular Junction--metabolism--ME; Neuromuscular Junction--physiology--PH; Patch-Clamp Techniques--methods --MT; Presynaptic Terminals--physiology--PH; Protein Structure, Tertiary --genetics--GE; Protein Structure, Tertiary--physiology--PH; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.; Structure-Activity Relationship; Temperature; Time Factors
CAS Registry No.: 0 (Drosophila Proteins); 0 (HSP40 Heat-Shock Proteins); 0 (Membrane Proteins); 0 (cysteine string protein); 7440-70-2 (Calcium)
Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)
Record Date Created: 20050304

Record Date Completed: 20060331

... protecting evoked release against thermal stress. Hence, CSP can also act as an Hsc70-independent **chaperone** protecting evoked release from thermal stress. Expression of mutant CSP lacking the L domain restored...
...; Expression--genetics--GE; HSP40 Heat-Shock Proteins--chemistry--CH; HSP40 Heat-Shock Proteins--genetics--GE; **Horseradish Peroxidase** --metabolism--ME; Humans; Immunohistochemistry--methods--MT; Membrane Potentials--physiology--PH; Membrane Proteins--chemistry--CH; Membrane...
Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)
Chemical Name: Drosophila Proteins; HSP40 Heat-Shock Proteins; Membrane Proteins; cysteine string protein; Calcium; **Horseradish Peroxidase**

6/9,K/54 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

20129747 PMID: 16213144
Development and optimization of a useful assay for determining Hsp90's inherent ATPase activity.
Avila Christopher; Kornilayev Boris A; Blagg Brian S J
The Department of Medicinal Chemistry, The University of Kansas, Malott 4070, Lawrence, 66045-7563, USA.
Bioorganic & medicinal chemistry (England) Feb 15 2006, 14 (4) p1134-42, ISSN 0968-0896--Print Journal Code: 9413298
Contract/Grant No.: R01 CA114393; CA; NCI; RR017708; RR; NCRR
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins. Inhibition of Hsp90 represents a promising approach towards the treatment of cancer because numerous signaling cascades can be simultaneously targeted by disruption of the Hsp90-mediated process. Hsp90's ATPase activity is essential to the Hsp90-mediated protein folding process, consequently, a coupled assay was developed and optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0.87 has been produced.
Descriptors: *Adenosine Triphosphate--metabolism--ME; *Adenosinetriphosphatase--metabolism--ME; *HSP90 Heat-Shock Proteins--metabolism--ME; Adenosine Triphosphate--pharmacology--PD; Adenosinetriphosphatase--analysis--AN; Adenosinetriphosphatase--antagonists and inhibitors--AI; Adenosinetriphosphatase--isolation and purification--IP; Catalysis; Glucose Oxidase--metabolism--ME; Glucosyltransferases--metabolism--ME; HSP90 Heat-Shock Proteins--analysis--AN; HSP90 Heat-Shock Proteins--antagonists and inhibitors--AI; HSP90 Heat-Shock Proteins--isolation and purification--IP; **Horseradish Peroxidase** --metabolism--ME; Hydrogen Peroxide--pharmacology--PD; Molecular Structure; Reproducibility of Results; Research Support, N.I.H., Extramural; Research Support, Non-U.S. Gov't; Temperature
CAS Registry No.: 0 (HSP90 Heat-Shock Proteins); 56-65-5 (Adenosine

Triphosphate); 7722-84-1 (Hydrogen Peroxide)
Enzyme No.: EC 1.1.3.4 (Glucose Oxidase); EC 1.11.1.- (**Horseradish Peroxidase**); EC 2.4.1.- (Glucosyltransferases); EC 2.4.1.8 (maltose phosphorylase); EC 3.6.1.3 (Adenosinetriphosphatase)
Record Date Created: 20060109
Record Date Completed: 20060317
Date of Electronic Publication: 20051004

The Hsp90 molecular **chaperone** is responsible for the conformational maturation of nascent polypeptides and the rematuration of denatured proteins...

... optimized for determination of Hsp90's inherent ATPase activity. Using maltose phosphorylase, glucose oxidase, and **horseradish peroxidase** as components of this assay, a highly reproducible assay with a Z-factor of 0 ...
...; Heat-Shock Proteins--antagonists and inhibitors--AI; HSP90 Heat-Shock Proteins--isolation and purification--IP; **Horseradish Peroxidase** --metabolism--ME; Hydrogen Peroxide--pharmacology--PD; Molecular Structure; Reproducibility of Results; Research Support, N.I...
Enzyme No.: EC 1.1.3.4 (Glucose Oxidase); EC 1.11.1.- (**Horseradish Peroxidase**); EC 2.4.1.- (Glucosyltransferases); EC 2.4.1.8 (maltose phosphorylase); EC 3.6...
Chemical Name: HSP90 Heat-Shock Proteins; Adenosine Triphosphate; Hydrogen Peroxide; Glucose Oxidase; **Horseradish Peroxidase** ; Glucosyltransferases; maltose phosphorylase; Adenosinetriphosphatase

6/9,K/55 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14590888 PMID: 14625857
Profiling of the cell surface proteome.
Jang Jun Ho; Hanash Samir
University of Michigan, Department of Pediatrics, Ann Arbor, MI 48109, USA.
Proteomics (Germany) Oct 2003, 3 (10) p1947-54, ISSN 1615-9853--Print Journal Code: 101092707
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The in depth-mining of the proteome necessitates the comprehensive analysis of proteins in individual subcellular compartments to uncover interesting patterns of protein expression that include assessment of protein location, trafficking and of post-translational modifications that are location specific. One of the compartments of substantial interest from a diagnostic and therapeutic point of view is the plasma membrane which contains intrinsic membrane proteins and other proteins expressed on the cell surface. Technologies are currently available for the comprehensive profiling of the cell surface proteome that rely on protein tagging of intact cells. Studies are emerging that point to unexpected patterns of expression of specific proteins on the cell surface, with a common occurrence of proteins previously considered to occur predominantly in

other compartments, notably the endoplasmic reticulum. The profiling of the cell surface and plasma membrane proteomes will likely provide novel insights and uncover disease related alterations.

Descriptors: *Membrane Proteins--analysis--AN; *Proteome--analysis--AN; *Proteomics--methods--MT; ADP-ribosyl Cyclase--analysis--AN; Antigens, CD --analysis--AN; Antigens, CD18--analysis--AN; Antigens, CD38; Avidin --chemistry--CH; Bacterial Proteins--chemistry--CH; Biotinylation; Carrier Proteins--analysis--AN; Catalase--analysis--AN; Cell Line, Tumor; Chromatography, Affinity; Electrophoresis, Gel, Two-Dimensional; HL-60 Cells; HLA-B Antigens--analysis--AN; HSP70 Heat-Shock Proteins--analysis --AN; Heat-Shock Proteins--analysis--AN; **Horseradish Peroxidase** --chemistry--CH; Humans; Membrane Glycoproteins; Membrane Proteins --isolation and purification--IP; Molecular Chaperones--analysis--AN; Polyvinyls--chemistry--CH; Protein Binding; Protein Disulfide-Isomerase --analysis--AN; Receptors, Transferrin--analysis--AN; Silver Staining; Spectrometry, Mass, Matrix-Assisted Laser Desorption-Ionization; U937 Cells

CAS Registry No.: 0 (Antigens, CD); 0 (Antigens, CD18); 0 (Bacterial Proteins); 0 (Carrier Proteins); 0 (HLA-B Antigens); 0 (HLA-Bw58); 0 (HSP70 Heat-Shock Proteins); 0 (Heat-Shock Proteins); 0 (Membrane Glycoproteins); 0 (Membrane Proteins); 0 (Molecular Chaperones); 0 (Polyvinyls); 0 (Proteome); 0 (Receptors, Transferrin); 0 (Strep-avidin conjugated horseradish peroxidase); 0 (glucose-regulated proteins); 0 (molecular chaperone GRP78); 1405-69-2 (Avidin); 24937-79-9 (polyvinylidene fluoride)

Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**); EC 1.11.1.6 (Catalase); EC 3.2.2.5 (ADP-ribosyl Cyclase); EC 3.2.2.5 (Antigens, CD38); EC 3.2.2.5 (CD38 protein, human); EC 5.3.4.1 (Protein Disulfide-Isomerase)

Record Date Created: 20031119

Record Date Completed: 20040723

...; B Antigens--analysis--AN; HSP70 Heat-Shock Proteins--analysis--AN; Heat-Shock Proteins--analysis--AN; **Horseradish Peroxidase** --chemistry --CH; Humans; Membrane Glycoproteins; Membrane Proteins--isolation and purification--IP; Molecular Chaperones--analysis--AN...

Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**); EC 1.11.1.6 (Catalase); EC 3.2.2.5 (ADP-ribosyl Cyclase); EC...

...Chemical Name: Shock Proteins; Membrane Glycoproteins; Membrane Proteins; Molecular Chaperones; Polyvinyls; Proteome; Receptors, Transferrin; Strep-avidin conjugated **horseradish peroxidase** ; glucose-regulated proteins; molecular **chaperone** GRP78; Avidin; polyvinylidene fluoride; **Horseradish Peroxidase** ; Catalase; ADP-ribosyl Cyclase; Antigens, CD38; CD38 protein, human; Protein Disulfide-Isomerase

6/9,K/56 (Item 5 from file: 155)

14187151 PMID: 12493773

Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone **function.**

Shin Bong Kyung; Wang Hong; Yim Anne Marie; Le Naour Francois; Brichory Franck; Jang Jun Ho; Zhao Rong; Puravs Eric; Tra John; Michael Claire W; Misek David E; Hanash Samir M

Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor, Michigan 48109-0656, USA.

Journal of biological chemistry (United States) Feb 28 2003, 278 (9) p7607-16, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print-Electronic
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS

There is currently limited data available pertaining to the global characterization of the cell surface proteome. We have implemented a strategy for the comprehensive profiling and identification of surface membrane proteins. This strategy has been applied to cancer cells, including the SH-SY5Y neuroblastoma, the A549 lung adenocarcinoma, the LoVo colon adenocarcinoma, and the Sup-B15 acute lymphoblastic leukemia (B cell) cell lines and ovarian tumor cells. Surface membrane proteins of viable, intact cells were subjected to biotinylation then affinity-captured and purified on monomeric avidin columns. The biotinylated proteins were eluted from the monomeric avidin columns as intact proteins and were subsequently separated by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained for the different cell populations analyzed. Identification of a subset of biotinylated proteins among the different cell populations analyzed using matrix-assisted laser desorption ionization and tandem mass spectrometry uncovered proteins with a restricted expression pattern in some cell line(s), such as CD87 and the activin receptor type IIB. We also identified more widely expressed proteins, such as CD98, and a sushi repeat-containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70, HSP60, HSP54, HSP27, and protein disulfide isomerase. Comprehensive profiling of the cell surface proteome provides an effective approach for the identification of commonly occurring proteins as well as proteins with restricted expression patterns in this compartment.

Descriptors: *Heat-Shock Proteins; *Molecular Chaperones--metabolism--ME; *Neoplasms--metabolism--ME; Activin Receptors, Type II--metabolism--ME; Amino Acid Sequence; Antigens, CD98--biosynthesis--BI; Biotinylation; Blotting, Western; Carrier Proteins--biosynthesis--BI; Electrophoresis, Gel, Two-Dimensional; HSP70 Heat-Shock Proteins--biosynthesis--BI; Humans; Microscopy, Fluorescence; Molecular Chaperones--biosynthesis--BI; Molecular Sequence Data; Oligonucleotide Array Sequence Analysis; Protein Array Analysis; Protein Binding; Protein Structure, Tertiary; Proteome; Receptors, Cell Surface--biosynthesis--BI; Spectrometry, Mass, Matrix-Assisted Laser Desorption-Ionization; Spectrum Analysis, Mass; Tumor Cells, Cultured

CAS Registry No.: 0 (Antigens, CD98); 0 (Carrier Proteins); 0 (HSP70 Heat-Shock Proteins); 0 (Heat-Shock Proteins); 0 (Molecular Chaperones); 0 (Proteome); 0 (Receptors, Cell Surface); 0 (molecular chaperone GRP78); 0 (plasminogen activator, urokinase receptors)

Enzyme No.: EC 2.7.1.- (activin receptor type II-B); EC 2.7.1.37 (Activin Receptors, Type II)

Record Date Created: 20030224
Record Date Completed: 20030408
Date of Electronic Publication: 20021218

... profiling of the cell surface proteome of cancer cells uncovers an

abundance of proteins with chaperone **function.**
... by two-dimensional PAGE, transferred to polyvinylidene difluoride membranes, and visualized by hybridization with streptavidin- **horseradish peroxidase** . Highly reproducible, but distinct, two-dimensional patterns consisting of several hundred biotinylated proteins were obtained...

... containing protein, a member of the selectin family. Remarkably, a set of proteins identified as **chaperone** proteins were found to be highly abundant on the cell surface, including GRP78, GRP75, HSP70...
...Chemical Name: Proteins; HSP70 Heat-Shock Proteins; Heat-Shock Proteins; Molecular Chaperones; Proteome; Receptors, Cell Surface; molecular **chaperone** GRP78; plasminogen activator, urokinase receptors; activin receptor type II-B; Activin Receptors, Type II

6/9,K/57 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

14180713 PMID: 12589809
Phospholipid assisted folding of a denatured heme protein: effect of phosphatidylethanolamine.
Debnath Dilip; Bhattacharya Shekhar; Chakrabarti Abhijit
Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, 700037, Kolkata, India.
Biochemical and biophysical research communications (United States) Feb 21 2003, 301 (4) p979-84, ISSN 0006-291X--Print Journal Code: 0372516
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
The role of the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins have not been done so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its refolding from denatured state. Dimyristoylphosphatidylethanolamine (DMPE), a bilayer-forming PE, was able to increase the reactivation yield of denatured HRP upon 30min refolding at 25 degrees C. However, dioleoylphosphatidylethanolamine (DOPE), containing one double bond in the fatty acid chains, which does not favour bilayer organization, did not support proper refolding. The phospholipids with N-methylated head groups, phosphatidylcholines, e.g., DMPC and DOPC showed differential effects when DMPC remained mostly non-supportive while DOPC on the contrary led to inhibition of the refolding of the denatured heme enzyme. Fluorescence spectroscopic studies also indicated changes in the microenvironments of the heme moiety and the single tryptophan residue of HRP in presence of the aminophospholipid.
Descriptors: ***Horseradish Peroxidase** --chemistry--CH; *Phosphatidylethanolamines--pharmacology--PD; Dimyristoylphosphatidylcholine--pharmacology--PD; Enzyme Reactivators--pharmacology--PD; Heme--chemistry--CH; Hemeproteins--chemistry--CH; Hemoglobins--chemistry--CH; **Horseradish Peroxidase** --drug effects--DE; **Horseradish Peroxidase** --metabolism--ME; In Vitro; Liposomes; Models, Chemical; Phosphatidylcholines

--pharmacology--PD; Phosphatidylethanolamines--metabolism--ME; Protein Denaturation; Protein Folding; Solubility; Spectrometry, Fluorescence
CAS Registry No.: 0 (1,2-dioleoyl-glycero-3-phosphatidyl ethanolamine); 0 (Enzyme Reactivators); 0 (Hemeproteins); 0 (Hemoglobins); 0 (Liposomes); 0 (Phosphatidylcholines); 0 (Phosphatidylethanolamines); 10015-85-7 (1,2-oleoylphosphatidylcholine); 13699-48-4 (Dimyristoylphosphatidylcholine); 14875-96-8 (Heme); 20255-95-2 (1,2-dimyristoylphosphatidylethanolamine)
Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)
Record Date Created: 20030218
Record Date Completed: 20030409

... the aminophospholipid, phosphatidylethanolamine (PE), has been well established to act as a non-protein molecular **chaperone** in the folding and assembly of polytopic membrane proteins. However, such studies with soluble proteins...

... so far and in particular with the heme proteins. We have used the heme enzyme, **horseradish peroxidase** (HRP), as the model heme protein and studied the effect of different phospholipids on its...
Descriptors: ***Horseradish Peroxidase** --chemistry--CH; *Phosphatidylethanolamines--pharmacology--PD...; pharmacology--PD; Enzyme Reactivators--pharmacology--PD; Heme--chemistry--CH; Hemeproteins--chemistry--CH; Hemoglobins--chemistry--CH; **Horseradish Peroxidase** --drug effects --DE; **Horseradish Peroxidase** --metabolism--ME; In Vitro; Liposomes; Models, Chemical; Phosphatidylcholines--pharmacology--PD; Phosphatidylethanolamines--metabolism--ME; Protein Denaturation...
Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)
...Chemical Name: Enzyme Reactivators; Hemeproteins; Hemoglobins; Liposomes; Phosphatidylcholines; Phosphatidylethanolamines; 1,2-oleoylphosphatidylcholine; Dimyristoylphosphatidylcholine; Heme; 1,2-dimyristoylphosphatidylethanolamine; **Horseradish Peroxidase**

6/9,K/58 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13176594 PMID: 11302741
Binding of a denatured heme protein and ATP to erythroid spectrin.
Chakrabarti A; Bhattacharya S; Ray S; Bhattacharyya M
Biophysics Division, Saha Institute of Nuclear Physics, 37 Belgachia Road, Calcutta, 700037, India. abhijit@biop.saha.ernet.in
Biochemical and biophysical research communications (United States) Apr 20 2001, 282 (5) p1189-93, ISSN 0006-291X--Print Journal Code: 0372516
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Spectrin is a large, worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of increasing concentrations of spectrin from that in the absence. This indicated that spectrin could bind denatured HRP and

inhibit their refolding. In presence of 1 mM ATP and 10 mM MgCl(2) the spectrin binding of denatured HRP is abolished. This activity of decreasing the reactivation yield was found to be ATP-dependent and the denatured enzyme after 30 min refolding in the presence of spectrin, pretreated with Mg/ATP, showed about 40% increase in the reactivation yield compared to the same in absence of spectrin. Fluorescence spectroscopic studies indicated binding of ATP to native spectrin showing concentration-dependent quenching of tryptophan fluorescence by ATP. The apparent dissociation constant of binding of ATP to spectrin was estimated to be 1.1 mM. A high affinity binding of spectrin with denatured HRP has been characterized (K(d) = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes. Copyright 2001 Academic Press.

Descriptors: *Adenosine Triphosphate--metabolism--ME; *Hemeproteins --metabolism--ME; * **Horseradish Peroxidase** --metabolism--ME; *Spectrin --metabolism--ME; Adenosine Triphosphate--chemistry--CH; Adenosine Triphosphate--pharmacology--PD; Animals; Binding, Competitive--physiology --PH; Dose-Response Relationship, Drug; Erythrocytes--chemistry--CH; Goats; Hemeproteins--chemistry--CH; **Horseradish Peroxidase** --chemistry--CH; Molecular Chaperones--metabolism--ME; Protein Binding--drug effects--DE; Protein Binding--physiology--PH; Protein Denaturation--drug effects--DE; Protein Denaturation--physiology--PH; Protein Folding; Research Support, Non-U.S. Gov't; Spectrin--chemistry--CH; Spectrometry, Fluorescence; Tryptophan

CAS Registry No.: 0 (Hemeproteins); 0 (Molecular Chaperones); 12634-43-4 (Spectrin); 56-65-5 (Adenosine Triphosphate); 73-22-3 (Tryptophan)

Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)

Record Date Created: 20010416

Record Date Completed: 20010517

...worm-like cytoskeletal protein that is abundant in all cell types. The denatured heme enzyme, **horseradish peroxidase** showed significant decrease in the reactivation yield, after 30 min of refolding, in presence of...

... characterized (K(d) = 16 nM). Since these properties are similar to those of established molecular **chaperone** proteins, these data indicate that spectrin might have a **chaperone** -like function in erythrocytes. Copyright 2001 Academic Press.

Descriptors: *Adenosine Triphosphate--metabolism--ME; *Hemeproteins --metabolism--ME; * **Horseradish Peroxidase** --metabolism--ME; *Spectrin --metabolism--ME...; Binding, Competitive--physiology--PH; Dose-Response Relationship, Drug; Erythrocytes--chemistry--CH; Goats; Hemeproteins --chemistry--CH; **Horseradish Peroxidase** --chemistry--CH; Molecular Chaperones--metabolism--ME; Protein Binding--drug effects--DE; Protein Binding--physiology--PH...

Enzyme No.: EC 1.11.1.- (**Horseradish Peroxidase**)

Chemical Name: Hemeproteins; Molecular Chaperones; Spectrin; Adenosine Triphosphate; Tryptophan; **Horseradish Peroxidase**

6/9,K/59 (Item 8 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

12425003 PMID: 10364256

Functional early endosomes are required for maturation of major histocompatibility complex class II molecules in human B lymphoblastoid cells.

Pond L; Watts C

Department of Biochemistry, Wellcome Trust Building, University of Dundee, Dundee DD1 4HN, United Kingdom.

Journal of biological chemistry (UNITED STATES) Jun 18 1999, 274 (25) p18049-54, ISSN 0021-9258--Print Journal Code: 2985121R

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must be degraded to enable peptide capture by MHC class II molecules. It remains controversial exactly which route or routes MHC class II/Ii complexes take to reach the sites of Ii processing and peptide loading. We have asked whether early endosomes are required for successful maturation of MHC class II molecules by using an in situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas transport of secretory Ig through the secretory pathway is virtually normal in the ablated cells, newly synthesized MHC class II/Ii complexes never reach compartments capable of processing Ii. These results strongly suggest that the transport of the bulk of newly synthesized MHC class II molecules through early endosomes is obligatory and that direct input into later endosomes/lysosomes does not take place.

Descriptors: *Antigens, Differentiation, B-Lymphocyte--immunology--IM; *B-Lymphocytes--immunology--IM; *Endosomes--metabolism--ME; *Histocompatibility Antigens Class II--immunology--IM; Antigens, Differentiation, B-Lymphocyte--metabolism--ME; Cell Line; Histocompatibility Antigens Class II--metabolism--ME; **Horseradish Peroxidase** ; Humans; Leupeptins --pharmacology--PD; Lysosomes--metabolism--ME; Microscopy, Fluorescence; Peroxidases; Precipitin Tests; Research Support, Non-U.S. Gov't; Transferrin

CAS Registry No.: 0 (Antigens, Differentiation, B-Lymphocyte); 0 (Histocompatibility Antigens Class II); 0 (Leupeptins); 0 (invariant chain); 11096-37-0 (Transferrin); 24365-47-7 (leupeptin)

Enzyme No.: EC 1.11.1. (Peroxidases); EC 1.11.1.- (**Horseradish Peroxidase**); EC 1.11.1.- (diaminobenzidine peroxidase)

Record Date Created: 19990715

Record Date Completed: 19990715

Major histocompatibility complex (MHC) class II molecules are targeted together with their invariant chain (Ii) **chaperone** from the secretory pathway to the endocytic pathway. Within the endosome/lysosome system, Ii must...

... situ peroxidase/diaminobenzidine compartment ablation technique. Cells whose early endosomes were selectively ablated using transferrin- **horseradish peroxidase** conjugates fail to mature their newly synthesized MHC class II molecules. We show that whereas...

; Antigens, Differentiation, B-Lymphocyte--metabolism--ME; Cell Line;

Histocompatibility Antigens Class II--metabolism--ME; **Horseradish Peroxidase** ; Humans; Leupeptins--pharmacology--PD; Lysosomes--metabolism --ME; Microscopy, Fluorescence; Peroxidases; Precipitin Tests; Research Support, Non...
Enzyme No.: EC 1.11.1. (Peroxidases); EC 1.11.1.- (**Horseradish Peroxidase**); EC 1.11.1.- (diaminobenzidine peroxidase)
Chemical Name: Antigens, Differentiation, B-Lymphocyte; Histocompatibility Antigens Class II; Leupeptins; invariant chain; Transferrin; leupeptin; Peroxidases; **Horseradish Peroxidase** ; diaminobenzidine peroxidase

6/9,K/60 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09576125 PMID: 8444889
Organelle-specific phosphorylation. Identification of unique membrane phosphoproteins of the endoplasmic reticulum and endosomal apparatus.
Rindress D; Lei X; Ahluwalia J P; Cameron P H; Fazel A; Posner B I; Bergeron J J
Department of Anatomy, McGill University, Montreal, Quebec, Canada.
Journal of biological chemistry (UNITED STATES) Mar 5 1993, 268 (7) p5139-47, ISSN 0021-9258--Print Journal Code: 2985121R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Highly purified endoplasmic reticulum fractions from rat liver and dog pancreas harbor membrane-associated kinases that phosphorylate integral membrane proteins of 90, 56, 35, and 15 kDa with [gamma-32P]GTP and of 90, 56, and 35 kDa with [gamma-32P]ATP. Of these, only the 35-kDa phosphoprotein was N-glycosylated. Screening of Golgi fractions, endosomes, plasma membranes, lysosomes, and mitochondria revealed phosphoproteins unique to each organelle. In particular, endosomes were found to harbor a 48-kDa extrinsic membrane protein and two or more integral membrane phosphoproteins of 30-35 kDa. None of these were N-glycosylated as judged by their insensitivity to digestion by N-glycosidase F and a lack of binding to concanavalin A or wheat germ agglutinin. Since the 30-35 kDa membrane phosphoproteins present in Golgi-free endosomal fractions were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique to endosomes. Since membrane phosphoproteins unique to the endoplasmic reticulum have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P.H., Ou, W.-J., Doherty, J.-J., II, Louvard, D., Bell, A.W., Dignard, D., Thomas, D.Y., and Bergeron, J.J.M. (1991) J. Biol. Chem. 266, 19599-19610; Ahluwalia, N., Bergeron, J.J.M., Wada, I., Degen, E., and Williams, D.B. (1992) J. Biol. Chem. 267, 10914-10918), then the unique endosomal phosphoproteins may serve equally important functions in addition to serving as novel markers for the organelle.
Descriptors: *Endoplasmic Reticulum--metabolism--ME; *Membrane Proteins --metabolism--ME; *Organelles--metabolism--ME; *Phosphoproteins--metabolism

--ME; Animals; Dogs; Golgi Apparatus--metabolism--ME; Intracellular Membranes--metabolism--ME; Liver--metabolism--ME; Pancreas--metabolism--ME; Phosphorylation; Protein Kinases--metabolism--ME; Rats; Research Support, Non-U.S. Gov't; Substrate Specificity
CAS Registry No.: 0 (Membrane Proteins); 0 (Phosphoproteins)
Enzyme No.: EC 2.7.1.37 (Protein Kinases)
Record Date Created: 19930406
Record Date Completed: 19930406

... were not detected in endosome-free, highly purified Golgi fractions and were found exclusively in **horseradish peroxidase** -containing endosomes as determined by the diaminobenzidine shift protocol, then these membrane phosphoproteins are unique...

... have been shown to have important functional significance in calcium binding and as a membrane **chaperone** (s) (Wada, I., Rindress, D., Cameron, P.H., Ou, W.-J., Doherty, J.-J., II...

6/9,K/61 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0378436 DBR Accession No.: 2005-24142 PATENT
New vectors having a nucleic acid encoding a heterologous recombinant fusion protein comprising an immunological molecule and a toxin, useful in research, diagnostic and/or therapeutic applications, such as in tumor disorders - recombinant vector-mediated gene transfer and expression in host cell
AUTHOR: MOTWANI N; BLACKBURN R
PATENT ASSIGNEE: APOLIFE INC 2005
PATENT NUMBER: US 20050191726 PATENT DATE: 20050901 WPI ACCESSION NO.: 2005-582224 (200559)
PRIORITY APPLIC. NO.: US 102000 APPLIC. DATE: 20050407
NATIONAL APPLIC. NO.: US 102000 APPLIC. DATE: 20050407
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A vector including one or more expression cassettes that comprise a nucleic acid encoding a heterologous recombinant fusion protein which comprises an immunological molecule and a toxin, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a method of producing a functional heterologous recombinant multichain or single-chain fusion protein, comprising transforming a Saccharomyces yeast host cell with a vector cited above, growing the transformed cell in culture to a stage of log phase growth, and inducing the cell culture to express the heterologous recombinant multichain or single-chain fusion protein, where the fusion protein comprises an immunotoxin, and where the immunotoxin comprises an antibody domain and an oxidase toxin domain; and (2) a vector comprising at least two expression cassettes, where a first expression cassette comprises a nucleic acid encoding a heterologous recombinant fusion protein that comprises an immunological molecule and a toxin, where a second expression cassette comprises a nucleic acid encoding an accessory molecule that is a **chaperone** protein selected from a BiP and a protein disulfide isomerase (PDI). BIOTECHNOLOGY - Preferred Vector: The expression cassettes in the vector cited above further comprise promoter sequences operatively linked to the nucleic acid encoding the

heterologous recombinant fusion protein, a translation initiation sequences, where the promoter sequences are located flush with and 5' to said translation initiation sequences, and a nucleic acid encoding a secretory signal sequence that is a human secretory signal sequence, yeast secretory sequence or secretory sequence native to the heterologous fusion protein. The vector further comprises an expression cassette comprising a nucleic acid encoding an accessory molecule that is a **chaperone** protein, or a BiP or PDI. A third expression cassette in the vector of (2) comprises a nucleic acid encoding an accessory molecule, where the accessory molecule is a **chaperone** protein selected from a BiP and PDI. Preferred Method: The yeast cell in producing a functional heterologous recombinant multichain or single-chain fusion protein is Saccharomyces cerevisiae. The strain of S. cerevisiae is Y112, Y113, Y114, Y115, Y116, Y117, Y118, Y119, Y120, Y121, Y122, Y123, Y124 or Y125. The yeast host cell comprises one or more mutations in glycosylation pathways. The recombinant fusion protein is glycosylated. The yeast host cell also comprises supersecretory activity. The transformed yeast cell is grown under fermentation parameters that improve the production of the recombinant fusion protein. The fermentation parameters are carbon sources, buffering systems, media formulations, vitamin levels, trace salt levels, temperature, aeration levels, oxygen levels, pH, induction time, and length of induction. The antibody domain comprises two chains, where one of the chains is operably linked to the toxin domain. One of the two chains is a heavy chain, where the other chain is a light chain. The vector further comprises multiple expression cassettes. The toxin is glucose oxidase, glucose-oxidase-related toxin, **horseradish peroxidase** , or **horseradish peroxidase** -related toxin. ACTIVITY - Cytostatic. No biological data given. MECHANISM OF ACTION - IgG-Agonist. USE - The methods and compositions of the present invention are useful in producing heterologous recombinant proteins for research, diagnostic and/or therapeutic applications, such as in tumor disorders.(40 pages)

DESCRIPTORS: recombinant heterologous fusion protein prep., vector-mediated gene transfer, expression in host cell, agonist, antibody, appl., cancer diagnosis, therapy tumor cytostatic (24, 39)

SECTION: THERAPEUTICS-Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Cancer-DIAGNOSTICS-Molecular Diagnostics

...ABSTRACT: a second expression cassette comprises a nucleic acid encoding an accessory molecule that is a **chaperone** protein selected from a BiP and a protein disulfide isomerase (PDI). BIOTECHNOLOGY - Preferred Vector: The...

... comprises an expression cassette comprising a nucleic acid encoding an accessory molecule that is a **chaperone** protein, or a BiP or PDI. A third expression cassette in the vector of (2) comprises a nucleic acid encoding an accessory molecule, where the accessory molecule is a **chaperone** protein selected from a BiP and PDI. Preferred Method: The yeast cell in producing a...

... vector further comprises multiple expression cassettes. The toxin is glucose oxidase, glucose-oxidase-related toxin, **horseradish peroxidase** , or **horseradish peroxidase** -related toxin. ACTIVITY - Cytostatic. No biological data given. MECHANISM OF ACTION - IgG-Agonist. USE - The...

6/9,K/62 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0338849 DBR Accession No.: 2004-11141 PATENT
Novel isolated denaturant which is e.g. boiling- or detergent-stable and/or protease resistant protein having chaperone **-like activity, useful for strengthening hair, nail or skin and for inducing wound healing - recombinant enzyme protein production for use in disease therapy and plant engineering**
AUTHOR: WANG W; PELAH D; ALEGRAND T; SHOSEYOV O; ALTMAN A; POUNY Y; MARTON I; WOLF A
PATENT ASSIGNEE: YISSUM RES DEV CO HEBREW UNIV JERUSALEM; FULCRUM SP LTD 2004
PATENT NUMBER: WO 200422697 PATENT DATE: 20040318 WPI ACCESSION NO.: 2004-248452 (200423)
PRIORITY APPLIC. NO.: US 233409 APPLIC. DATE: 20020904
NATIONAL APPLIC. NO.: WO 2003IL723 APPLIC. DATE: 20030902
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) having **chaperone** -like activity and **horseradish peroxidase** (HRP) protection activity, as determined by HRP protection assay, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid (II) comprising a first polynucleotide encoding (I), and a second polynucleotide including a promoter sequence operably linked to the first polynucleotide for directing an expression of (I); (2) a nucleic acid construct comprising (II); (3) a cell transformed with (II); (4) an organism transformed with (II); (5) isolating a gene encoding (I) from a biological source, involves screening an expression library with a polynucleotide encoding (I); (6) an antibody (III) recognizing one or more epitopes of (I); (7) enriching or isolating (M1) denaturant stable (boiling stable or detergent stable) and/or protease resistant protein from a biological source, involves extracting total proteins from the biological source to obtain a protein extract, boiling the protein extract, collecting the soluble proteins and optionally assaying for **chaperone** -like activity of the soluble proteins and enriching or isolating the stable protein having **chaperone** -like activity; (8) isolating a gene encoding (I) from a biological source; (9) identifying a nucleic acid potentially encoding (I), involves searching an electronic library containing several nucleic acid and/or amino acid sequences for sequences having a predetermined degree of identity or homology to any one of the nucleic acid sequences (N1) chosen from 5 fully defined sequences of 567, 593, 357, 497, 366 base pairs as given in the specification, or to any one of the amino acid sequences (A1) chosen from 26 fully defined sequences e.g., 108, 98, 98, 98, 84, 98, 98, 109, 47, 98, 98, 93 and 108 amino acids as given in the specification, or their portions or corresponding to at least 15 bases; (10) isolating a nucleic acid potentially encoding (I); (11) detergent-free isolation of a protease-resistant protein having **chaperone** -like activity from a biological source, involves extracting total proteins from the biological source, to obtain a protein extract, contacting the protein extract with a protease, and isolating a protease-resistant protein, and optionally assaying the protease-resistant protein for **chaperone**

-like activity; (12) a fusion protein (IV) comprising (I) fused to an additional polypeptide; (13) a transgenic plant expressing (I) above a natural amount of (I) in the plant; (14) rendering a plant more tolerant to a biotic or abiotic stress, involves engineering the plant to express (I) above a natural amount of (I) in the plant; (15) rendering a plant more recoverable from a biotic or abiotic stress, involves engineering the plant to express (I) above a natural amount of (I) in the plant; (16) isolating (M2) a boiling stable protein from a biological source, involves carrying out extracting, and boiling steps of (M1), recovering soluble protein fraction, and optionally assaying the protease resistant protein for **chaperone** -like activity; (17) a pharmaceutical composition (V) comprising (I) as an active ingredient and a carrier; (18) a hetero complex (VI) comprising an oligomer including several of (I), and at least two different molecules being fused to the oligomer; and (19) increasing a specific activity of a pre-isolated (I) as determined in Units of protecting activity per mg protein, involves autoclaving the pre-isolated (I), or treating the pre-isolated (I) with a protease. BIOTECHNOLOGY - Preferred Protein: The isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) has **chaperone** -like activity and **horseradish peroxidase** (HRP) protection activity, as determined by HRP protection assay, of at least 10 Units/mg protein, where the HRP protection assay comprises mixing (I) at different final protein concentrations at a predetermined volume with 100 microliters of 5 nM HRP present in 40 mM N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid (HEPES) buffer at pH 7.5, thus forming a first reaction mixture, and following incubation of the reaction mixture at 25 degreesC for 16 hours, determining HRP remaining enzymatic activity by mixing 5 microliters of the first reaction mixture with 100 microliters of 3,3',5,5'-tetramethylbenzidine, thus forming a second reaction mixture, incubating the second reaction mixture for 10 minutes, stopping a reaction of the second reaction mixture by an addition of 100 microliters of 1 M sulfuric acid and recording colorimetric change in the second reaction mixture at 435 nm, and units are defined as a dilution factor of (I) at a concentration of 1 mg/ml that confers 50% protection of HRP activity in the HRP protection assay. In (I), the HRP protection activity is of 15000, 10000, 8000, 6000, 5500, 5000, 4500, 4000, 3500, 3000, 2500, 2000, 1500, 1000 or at least 500 Units/mg protein. Preferred Nucleic Acid: In (II), the promoter sequence is a eukaryotic constitutive promoter. The promoter is a plant promoter chosen from a constitutive plant promoter, a tissue specific plant promoter and an inducible plant promoter. The constitutive plant promoter is chosen from Cauliflower mosaic virus (CaMV)35S plant promoter, CamV19S plant promoter, figwort mosaic virus (FMV)34S plant promoter, sugarcane bacilliform badnavirus plant promoter, CsVMV plant promoter, Arabidopsis ACT2/ACT8 actin plant promoter, Arabidopsis ubiquitin UBQ1 plant promoter, barley leaf thionin BTH6 plant promoter, and rice actin plant promoter. The tissue specific plant promoter is chosen from bean phaseolin storage protein plant promoter, DLEC plant promoter, PHSbeta plant promoter, zein storage protein plant promoter, conglutin gamma plant promoter from soybean, AT2S1 gene plant promoter, ACT11 actin plant promoter from Arabidopsis, napA plant promoter from Brassica napus and potato patatin gene plant promoter. The inducible plant promoter is chosen from a light-inducible plant promoter derived from the pea rbcS gene, a plant promoter from the alfalfa rbcS gene, DRE, MYC and MYB plant promoters, which are active in drought, INT, INPS, prxEa, Ha hsp17.7G4 and RD21 plant promoters active in high

salinity and osmotic stress, and hsr203J and str246C plant promoters active in pathogenic stress. The first polynucleotide has a sequence at least 60% identical with 6 fully defined sequences of 567, 593, 357, 428, 497 or 366 base pairs as given in the specification, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap weight equals 50, length weight equals 3, average match equals 10 and average mismatch equals -9. (I) Has a sequence at least 60% identical to a fully defined sequence of 108 or 112 amino acids as given in the specification, as determined using the BestFit software of the Wisconsin sequence analysis package, utilizing the Smith and Waterman algorithm, where gap creation penalty equals 8 and gap extension penalty equals 2. (I) Is natively an oligomer. The **chaperone** -like activity includes heat stabilization of proteins. (II) Further comprises a third polynucleotide encoding an additional protein, and being adjacent and in frame to the first polynucleotide, where the first and third polynucleotides encoding, in combination, a fusion protein of the (I) and the additional protein. Preferred Pharmaceutical Composition: (V) Is packaged in a package and identified in print for use in a wound healing application or strengthening and/or grooming hair, nail or skin application. Preferred Method: Isolating a gene encoding (I) from a biological source, comprises: (a) carrying out the steps of extracting, boiling, collecting, assaying, and isolating of (M1), raising antibodies recognizing the stable protein having **chaperone** -like activity and screening an expression library with the antibodies; or (b) carrying out the steps of (M1), micro-sequencing the stable protein to obtain at least a partial amino acid sequence, designing an oligonucleotide corresponding to the amino acid sequence and screening a library with the oligonucleotide. Isolating a nucleic acid potentially encoding (I) comprises: (a) screening a cDNA or genomic library with a polynucleotide of at least 17 bases and at least 60% identical to a contiguous portion of 6 fully defined sequences of 567, 593, 357, 428, 497 or 366 base pairs as given in the specification; or (b) providing at least one pair of oligonucleotides each being at least 15 bases in length, including one or more oligonucleotides corresponding to (N1), and selected for amplifying a nucleic acid having a degree of identity with, or encoding proteins exhibiting homology to (A1), contacting at least one pair of oligonucleotides with a sample of nucleic acid and amplifying the nucleic acid having degree of identity with, or encoding proteins exhibiting homology to (A1), and using nucleic acid having degree of identity with, or encoding proteins exhibiting homology to (A1) for isolating a nucleic acid potentially encoding (I). (M2) further involves digesting the protein extract with a protease. Preferred Hetero Complex: In (VI), the two different molecules comprise a first enzyme and a second enzyme, that catalyze sequential or different reactions in a synthesis or degradation pathway. The two different molecules comprise at least a binding molecule and a reporter molecule. Preferred Fusion Protein: (I) Is fused to the additional polypeptide through a peptide bond or a cross-linker, where (IV) has an oligomeric form. ACTIVITY - Vulnerary; Nootropic; Neuroprotective. No biological data given. MECHANISM OF ACTION - Prevents aggregation of aggregating proteins; Inducer of immune response (claimed). USE - (I) Is useful for preventing an aggregating protein from aggregating into an aggregate, which involves causing an effective amount of (I) to become in contact with aggregating protein. (I) Is useful for de-aggregating aggregates of an aggregating protein, which involves causing an effective amount of (I)

to become in contact with the aggregate. (I) Is useful for stabilizing a protein against denaturing conditions, which involves causing an effective amount of (I) to become in contact with the protein. (I) Is useful for protecting an enzyme preparation from reduction in enzymatic activity, which involves adding (I) to the enzyme preparation in an amount sufficient for protecting the enzyme preparation from reduction in enzymatic activity. (I) is useful for repairing at least a portion of lost enzymatic activity of an enzyme preparation, which involves adding (I) to the enzyme preparation, in an amount sufficient for repairing portion of the lost enzymatic activity of the enzyme preparation. (I) is useful for increasing cell migration, which involves exposing the cells to (I), in an amount sufficient for increasing cell migration. (I) is useful for accelerating or inducing wound healing, which involves administering (I) on to a wound, in an amount sufficient for accelerating or inducing wound healing. (I) is useful for strengthening or grooming hair, nail or skin, which involves administering (I) onto the hair, nail or skin sufficient for strengthening or grooming the hair, nail or skin. (I) is useful for treating a disease associated with protein aggregation of an aggregating protein, which involves administering (I) to a subject who is in need, in an amount sufficient for de-aggregating and/or preventing aggregation of the aggregating protein such as beta-amyloid or prion. (I) is useful for increasing a binding avidity of a binding molecule, which involves displaying multiple copies of the binding molecule on a surface of an oligomer of (I), where the binding molecule is chosen from a receptor, ligand, enzyme, substrate, inhibitor, antibody or antigen. (I) is useful in administering a polypeptide to an animal having a immune system by reducing an immune response against the polypeptide, which involves administering the polypeptide being fused to (I), to the animal and thus reducing the immune response against the polypeptide as compared to the immune response that is developed by administering the polypeptide alone to the animal. (III) is useful for isolating gene encoding (I), which involves screening an expression library with (III). (IV) is useful in immunization, which involves subjecting an immune system of a mammal to (IV) (claimed). (I) is useful for treating a disease such as Alzheimer's disease and prion associated diseases e.g., encephalus spongyform, by preventing aggregation of aggregating proteins. ADVANTAGE - (I) retains its activity and oligomerability also when forming a fusion protein. EXAMPLE - Boiling stable protein fractions of aspen, tomato M82, VF36 and pine were prepared as follows: Crude plant extracts were centrifuged for 10 minutes and supernatants were transferred to fresh tubes. The supernatants were subjected to a 10-minutes boiling session, then kept on ice for 5 minutes and centrifuged for 10 minutes. Resulting supernatants were precipitated by adding 4 volumes of cold acetone, and centrifuged for 10 minutes. Boiling stable proteins were then recovered by dissolving the pellets in 10 mM Tris-hydrochloric acid buffer (pH 7.5). The total boiling-stable proteins were separated on a 17% sodium dodecyl sulfate (SDS)-tricine polyacrylamide gel electrophoresis (PAGE), during which two bands of 66 and 116 kDa band were obtained. The 66 kDa band was found to represent a germin-like protein. Acetone-precipitated boiling-stable proteins of aspen plant were dissolved in 1X tricine-SDS sample buffer (100 mM Tris-hydrochloric acid pH 6.8, 20% glycerol, 1% SDS, 0.025% Coomassie blue), and then separated on a preparative 17% polyacrylamide tricine-SDS gel. Major bands corresponding to stable proteins (SP)-1 (116 kDa oligomer and 12.4 kDa monomer) protein were excised from the

gel. SP1 oligomer and monomer were electro-eluted separately, in a dialysis bag. The eluted product was further dialyzed against 500 volumes of 10 mM Tris-hydrochloric acid overnight at 4degreesC, followed by acetone precipitation and centrifugation. Purified SP1 was obtained by dissolving the pellet in 10 mM Tris-hydrochloric acid. The prepared stable protein when maintained with **horseradish peroxidase** , was found to maintain its activity. (176 pages)

DESCRIPTORS: recombinant denaturant stable protease resistant, **chaperone** -like protein, **horseradish peroxidase** , prep., vector-mediated gene transfer expression in host cell, antibody, fusion protein, appl. transgenic plant construction, biotic, abiotic stress tolerance, aggregating protein prevention, protein stabilization, enzyme loss act. repair, cell migration, wound healing induction, grooming hair, Alzheimer disease, prion associated disease, encephalus spongyform therapy plant Armoracia rusticana enzyme EC-1.11.1.7 crop improvement vulnerary nootropic neuroprotective DNA sequence protein sequence (23, 22)

SECTION: THERAPEUTICS-Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Central Nervous System-AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering; DISEASE-Other Diseases

...**denaturant which is e.g. boiling- or detergent-stable and/or protease resistant protein having** chaperone **-like activity, useful for strengthening hair, nail or skin and for inducing wound healing - recombinant...**

...ABSTRACT: isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) having **chaperone** -like activity and **horseradish peroxidase** (HRP) protection activity, as determined by HRP protection assay, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS...

... a protein extract, boiling the protein extract, collecting the soluble proteins and optionally assaying for **chaperone** -like activity of the soluble proteins and enriching or isolating the stable protein having **chaperone** -like activity; (8) isolating a gene encoding (I) from a biological source; (9) identifying a...

... nucleic acid potentially encoding (I); (11) detergent-free isolation of a protease-resistant protein having **chaperone** -like activity from a biological source, involves extracting total proteins from the biological source, to...

... protease, and isolating a protease-resistant protein, and optionally assaying the protease-resistant protein for **chaperone** -like activity; (12) a fusion protein (IV) comprising (I) fused to an additional polypeptide; (13...

... steps of (M1), recovering soluble protein fraction, and optionally assaying the protease resistant protein for **chaperone** -like activity; (17) a pharmaceutical composition (V) comprising (I) as an active ingredient and a...

... isolated denaturant stable (boiling stable or detergent stable) and/or protease resistant protein (I) has **chaperone** -like activity and **horseradish peroxidase** (HRP) protection activity, as determined by HRP protection assay, of at least 10 Units/mg...

... penalty equals 8 and gap extension penalty equals 2. (I) Is natively an oligomer. The **chaperone** -like activity includes heat stabilization of proteins. (II) Further comprises a third polynucleotide encoding an...

... extracting, boiling, collecting, assaying, and isolating of (M1), raising antibodies recognizing the stable protein having **chaperone** -like activity and screening an expression library with the antibodies; or (b) carrying out the...

... the pellet in 10 mM Tris-hydrochloric acid. The prepared stable protein when maintained with **horseradish peroxidase** , was found to maintain its activity. (176 pages)
DESCRIPTORS: recombinant denaturant stable protease resistant, **chaperone** -like protein, **horseradish peroxidase** , prep., vector-mediated gene transfer expression in host cell, antibody, fusion protein, appl. transgenic plant...

6/9,K/63 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0329626 DBR Accession No.: 2004-01918 PATENT
New hybrid protein chaperone **(e.g. heat shock protein) useful for stabilizing proteins and/or protein activities, or as an agent to prevent protein aggregation, or for treating diseases involving altered protein conformations - involving vector-mediated gene transfer and expression in host cell for use in gene therapy**
AUTHOR: QUINLAN R
PATENT ASSIGNEE: UNIV DUNDEE 2003
PATENT NUMBER: WO 200391266 PATENT DATE: 20031106 WPI ACCESSION NO.: 2003-865571 (200380)
PRIORITY APPLIC. NO.: GB 20029334 APPLIC. DATE: 20020423
NATIONAL APPLIC. NO.: WO 2003GB1721 APPLIC. DATE: 20030423
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A hybrid protein **chaperone** for stabilizing proteins and/or protein activities, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a stabilized protein formulation comprising at least one protein associated with the above hybrid protein **chaperone** ; (2) a method for stabilizing proteins and protein stabilities in an aqueous solution, comprising adding the above hybrid protein **chaperone** to the aqueous solution; (3) a method for stabilizing insulin in an aqueous solution, comprising adding HSP17.5, alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 to the solution; (4) a method for stabilizing citrate synthase in an aqueous solution, comprising adding alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 to the solution; (5) a method for stabilizing luciferase in an aqueous solution, comprising adding HSP17.5, alpha-crystallin or HSP27 to the solution; (6) a method for stabilizing **horseradish peroxidase** (HRP) conjugate in an aqueous solution, comprising adding alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 to the solution; (7) a method for stabilizing an antibody or its fragment or conjugate in an aqueous solution, comprising adding HSP27 to the aqueous solution; (8) a method for stabilizing an expressed recombinant protein, comprising providing a cell capable of expressing the recombinant protein and the hybrid protein **chaperone** , and expressing

the recombinant protein and the hybrid protein **chaperone** ; (9) a cell capable of expressing a recombinant protein and the hybrid protein **chaperone** ; (10) a nucleic acid sequence capable of encoding the hybrid protein **chaperone** ; and (11) a vector comprising the above nucleic acid sequence. BIOTECHNOLOGY - Preferred Protein **Chaperone** : The hybrid is a macromolecule composed of two or more portions of different origins. The portion is a region of protein or nucleic acid sequence encoding a structural domain of a protein **chaperone** or its functional homologue. The structural domain is a central domain, N- or C-terminal region of a protein **chaperone** or its functional homologue. The protein **chaperone** is a heat shock protein (HSP) selected from HSP90, HSP70 and HSP60. The protein **chaperone** is a small HSP selected from alphaA-crystallin, alphabeta-crystallin, HSP27, HSP20, MKBP, HSPB3, HSPB4, HSPB5, cvHSP, HSPB8 and HSPB9. The portions comprise sub-domains or residues of sHSP. The residue is Arginine 120. The sub-domain is C-terminal region. A potion of the **chaperone** is replaced with a similar portion from a **chaperone** of a different origin. The C-terminal portion of alphaB-crystallin is replaced with a C-terminal portion of HSP27 (alphaB-HSP27). The alphaB-HSP27 comprises the N-terminus and central portion of alphaB-crystallin and C-terminal tail of HSP27. Preferred Formulation: The ratio of protein to hybrid protein **chaperone** in the formulation is in the region of 25:1 to 1:100 (preferably 1:0.0625 to 1:40). Preferred Method: In stabilizing proteins and protein stabilities in an aqueous solution, the protein to be stabilized is an enzyme, therapeutic protein, diagnostic protein, antibody, antibody fragment or antibody conjugate. The protein is homocysteine desulphurase. The antibody conjugate is linked to an enzyme reporter. The enzyme reporter is HRP, alkaline phosphatase (ALP) or luciferase. The stabilizing is the prevention or arresting of the unfolding process and preservation of protein activity/function. The preservation of protein activity/function is achieved by assisting proteins to fold correctly and maintaining the proteins in a folded conformation. The hybrid protein **chaperone** is alphaB-crystallin and the protein is luciferase. The **chaperone** is alphaB-HSP27 and the protein is insulin, HRP conjugate or luciferase. In stabilizing insulin in an aqueous solution, the HSP17.5 is used to stabilize insulin at 37degreesC, and the HSP27 or alpha-crystallin is used to stabilize insulin at 44degreesC. In stabilizing citrate synthase in an aqueous solution, the alpha-crystallin is used to stabilize citrate synthase at 50degreesC. In stabilizing luciferase in an aqueous solution, the HSP17.5 or alphaB-crystallin is used to stabilize luciferase at room temperature. In stabilizing HRP conjugate in an aqueous solution, the HSP27 and HSP25 are used to stabilize HRP conjugate at room temperature. Alternatively, the HSP27, alphaB-crystallin or HSP25 is used to stabilize HRP conjugate at 37degreesC. In stabilizing an antibody or its fragment or conjugate in an aqueous solution, the HSP27 is used to stabilize an antibody at room temperature.Preferred Vector: The vector further comprises a nucleic acid capable of encoding a recombinant protein intended to be stabilized by the hybrid protein **chaperone** . The recombinant protein is a therapeutically important protein. ACTIVITY - Cardiant; Ophthalmological; Neuroprotective. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The hybrid protein **chaperone** is useful as an agent to prevent protein aggregation, as an inhibitor of cell death and genome stability pathways, for identification of proteins that are in the process of unfolding, for the treatment of diseases involving altered protein conformations (e.g. cardiomyopathies, cataract or neurodegenerative

disease), or for the manufacture of a medicament for the treatment of the diseases mentioned above. The HSP17.5, alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin or HSP25 is used for stabilizing insulin, citrate synthase or HRP conjugate. The HSP17.5, alpha-crystallin or HSP27 may also be used for stabilizing luciferase. In addition, HSP27 may be used for stabilizing an antibody or its fragment or conjugate (all claimed). (45 pages)

DESCRIPTORS: hybrid recombinant protein prep., isol., vector-mediated gene transfer, expression in host cell, appl. cardiomyopathy, neurodegenerative disease therapy, gene therapy DNA sequence protein sequence (23, 04)

SECTION: THERAPEUTICS-Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Cardiovascular-DISEASE-Central Nervous System; THERAPEUTICS-Gene Therapy

New hybrid protein chaperone **(e.g. heat shock protein) useful for stabilizing proteins and/or protein activities, or as...**

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A hybrid protein **chaperone** for stabilizing proteins and/or protein activities, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also...

... a stabilized protein formulation comprising at least one protein associated with the above hybrid protein **chaperone** ; (2) a method for stabilizing proteins and protein stabilities in an aqueous solution, comprising adding the above hybrid protein **chaperone** to the aqueous solution; (3) a method for stabilizing insulin in an aqueous solution, comprising...

... adding HSP17.5, alpha-crystallin or HSP27 to the solution; (6) a method for stabilizing **horseradish peroxidase** (HRP) conjugate in an aqueous solution, comprising adding alpha-crystallin, HSP27, alphaB-crystallin, alphaA-crystallin...

... protein, comprising providing a cell capable of expressing the recombinant protein and the hybrid protein **chaperone** , and expressing the recombinant protein and the hybrid protein **chaperone** ; (9) a cell capable of expressing a recombinant protein and the hybrid protein **chaperone** ; (10) a nucleic acid sequence capable of encoding the hybrid protein **chaperone** ; and (11) a vector comprising the above nucleic acid sequence. BIOTECHNOLOGY - Preferred Protein **Chaperone** : The hybrid is a macromolecule composed of two or more portions of different origins. The...

... a region of protein or nucleic acid sequence encoding a structural domain of a protein **chaperone** or its functional homologue. The structural domain is a central domain, N- or C-terminal region of a protein **chaperone** or its functional homologue. The protein **chaperone** is a heat shock protein (HSP) selected from HSP90, HSP70 and HSP60. The protein **chaperone** is a small HSP selected from alphaA-crystallin, alphabeta-crystallin, HSP27, HSP20, MKBP, HSPB3, HSPB4...

... residue is Arginine 120. The sub-domain is C-terminal region. A potion of the **chaperone** is replaced with a similar portion from a **chaperone** of a different origin. The C-terminal portion of alphaB-crystallin is replaced with a...

... and C-terminal tail of HSP27. Preferred Formulation: The ratio of protein to hybrid protein **chaperone** in the formulation is in the region of 25:1 to 1:100 (preferably 1...

... proteins to fold correctly and maintaining the proteins in a folded conformation. The hybrid protein **chaperone** is alphaB-crystallin and the protein is luciferase. The **chaperone** is alphaB-HSP27 and the protein is insulin, HRP conjugate or luciferase. In stabilizing insulin ...

...acid capable of encoding a recombinant protein intended to be stabilized by the hybrid protein **chaperone** . The recombinant protein is a therapeutically important protein. ACTIVITY - Cardiant; Ophthalmological; Neuroprotective. No biological data given. MECHANISM OF ACTION - Gene therapy. USE - The hybrid protein **chaperone** is useful as an agent to prevent protein aggregation, as an inhibitor of cell death...

6/9,K/64 (Item 4 from file: 357)

0326299 DBR Accession No.: 2003-27440 PATENT

Vector including one or more expression cassettes useful for producing functional heterologous recombinant multichain fusion protein comprising immunological molecule and toxin - recombinant protein production via plasmid expression in host cell

AUTHOR: MOTWANI N; BLACKBURN R

PATENT ASSIGNEE: APOLIFE INC 2003

PATENT NUMBER: US 20030100112 PATENT DATE: 20030529 WPI ACCESSION NO.: 2003-777314 (200373)

PRIORITY APPLIC. NO.: US 172867 APPLIC. DATE: 20020619

NATIONAL APPLIC. NO.: US 172867 APPLIC. DATE: 20020619

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A vector (I) including one or more expression cassettes where one of the expression cassettes comprises a nucleic acid encoding a heterologous recombinant fusion protein which comprises an immunological molecule such as single chain antibody ScFv and a toxin such as glucose oxidase. BIOTECHNOLOGY - Preferred Vector: In (I), the expression cassettes further comprises promoter sequences operatively linked to the nucleic acid encoding the heterologous recombinant fusion protein. The expression cassettes further comprise a translation initiation sequences and where the promoter sequences are located 5' to the translation initiation sequences. The expression cassettes further comprise a nucleic acid encoding a secretory signal sequence. The secretory signal sequence is chosen from human secretory signal sequences, yeast secretory sequences and secretory sequences which are native to the heterologous fusion protein. The first expression cassette includes a nucleic acid encoding a heterologous recombinant fusion protein which comprises an immunological molecule and a toxin, where the immunological molecule is a first chain of an immunological molecule and the toxin is glucose oxidase, and the second expression cassette includes a nucleic acid encoding a heterologous recombinant fusion protein which comprises an immunological molecule and a toxin where the immunological molecule is a second chain of an immunological molecule and the toxin is **horseradish peroxidase** . The

first chain is heavy chain and the second chain is light chain or vice versa. (I) further includes an expression cassette comprising a nucleic acid encoding an accessory molecule. The accessory molecule is a **chaperone** protein. The **chaperone** protein is chosen from BiP and protein disulfide isomerase (PDI). USE - (I) is useful for producing a functional heterologous recombinant multichain fusion protein comprising an immunological molecule and a toxin which involves transforming a yeast host cell with (I), growing the transformed yeast cell in culture to a stage of log phase growth, and inducing the yeast cell culture to express the recombinant fusion protein. The yeast cell is Saccharomyces cerevisiae. The strain of S.cerevisiae is chosen from Y112, Y113, Y114, Y115, Y116, Y117, Y118, Y119, Y120, Y121, Y122, Y123, Y124, and Y125. The yeast cell comprises one or more mutations in glycosylation pathways. The recombinant fusion protein is glycosylated. The yeast cell comprises supersecretory activity. The yeast cell overexpresses catalase. The transformed yeast cell is grown under altered fermentation parameters which improve the production of the recombinant fusion protein. The altered fermentation parameters are chosen from carbon sources, buffering systems, media formulations, vitamin levels, trace salt levels, temperature, aeration levels, oxygen levels, pH, induction time and length of induction. The vitamin level is altered by the addition of biotin. The immunological molecule comprises two chains and one of the chains is fused to the toxin. The two chains are heavy and light chain and where the toxin is chosen from glucose oxidase, glucose-oxidase-related toxin, **horseradish peroxidase** and **horseradish peroxidase** -related toxin. The vector comprises multiple expression cassettes (all claimed). ADVANTAGE - (I) is cost effective and allows efficient production of heterologous recombinant fusion protein. EXAMPLE - The Fv region of pSPORT-FvSA1 template of Escherichia coli vector was amplified by PCR using oligonucleotide primers which introduced a 5' EcoRI restriction site, and a 3' BamHI site into the PCR product. The glucose oxidase coding region was obtained by PCR using a plasmid template containing a genomic insert from Aspergillus niger and primers which incorporated a 5' BamHI site, and a 3' NotI site into the amplified sequences. The FvSA and glucose oxidase (GO) fragments were cloned by three-way ligation into a pBluescript plasmid containing the yeast expression cassette. EcoRI and NotI restriction sites located between the secretory and termination sequences were utilized to insert the immunotoxin components in frame. The resultant plasmid is (SK+(GO Fusion)). The complete expression cassette was removed from the plasmid through restriction digestion with KpnI and SphI, and cloned by blunt-end ligation into either or both of the unique BamHI and HpaI sites in the pPM40 yeast shuttle vector.(40 pages)

DESCRIPTORS: recombinant multichain glycosylated fusion protein prep., single chain antibody, vector plasmid pPM40-mediated gene transfer expression in Saccharomyces cerevisiae host cell culture fermentation, promoter sequence, translation initiation sequence, human, yeast secretory signal sequence, glucose-oxidase, **horseradish peroxidase** animal mammal enzyme EC-1.1.3.4 plant Armoracia rusticana EC-1.11.1.7 yeast fungus strain improvement (22, 50)

SECTION: GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis-THERAPEUTICS-Protein Therapeutics

...ABSTRACT: the immunological molecule is a second chain of an immunological molecule and the toxin is **horseradish peroxidase** . The first chain is heavy chain and the second chain is light chain or vice ...

... expression cassette comprising a nucleic acid encoding an accessory molecule. The accessory molecule is a **chaperone** protein. The **chaperone** protein is chosen from BiP and protein disulfide isomerase (PDI). USE - (I) is useful for...

... light chain and where the toxin is chosen from glucose oxidase, glucose-oxidase-related toxin, **horseradish peroxidase** and **horseradish peroxidase** -related toxin. The vector comprises multiple expression cassettes (all claimed). ADVANTAGE - (I) is cost effective ...

DESCRIPTORS: ...cell culture fermentation, promoter sequence, translation initiation sequence, human, yeast secretory signal sequence, glucose-oxidase, **horseradish peroxidase** animal mammal enzyme EC-1.1.3.4 plant Armoracia rusticana EC-1.11.1...

6/9,K/65 (Item 5 from file: 357)

0263436 DBR Accession No.: 2001-03012

Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb protein - using plasmid pTrc-OmpA-HRP and expression of folding accessory proteins**

AUTHOR: Kondo A; Kohda J; Endo Y; Shiromizu T; Kurokawa Y; Nishihara K ; Yanagi H; Yura T; Fukuda H
CORPORATE AFFILIATE: Univ.Kobe HSP-Res.Inst.Kyoto
CORPORATE SOURCE: Department of Chemical Science and Engineering, Faculty of Engineering, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Hyogo 657-8501, Japan.

JOURNAL: J.Ferment.Bioeng. (90, 6, 600-06) 2000
ISSN: 1389-1723 CODEN: JFBIEX
LANGUAGE: English

ABSTRACT: The effect of folding accessory proteins, molecular chaperones and foldases on the productivity of active **horseradish peroxidase** (HRP, EC-1.11.1.7) was studied in Escherichia coli JM109. Plasmid pTrc-OmpA-HRP expressing HRP and plasmids expressing the various folding accessory proteins were transformed in JM109. The cells were cultured at 37 deg in Luria-Bertani medium for 30 min then induced with different concentrations of IPTG. Induction with 1 uM IPTG produced HRP, whereas induction with 1 mM IPTG produced only tiny amounts. Expression of foldases DsbA-DsB or DsbC-DsbD increased the production of HRP but the expression of chaperones did not improve production of HRP. In a single HRP expression system, the growth of E. coli was significantly inhibited by 1 mM IPTG, but when HRG was expressed with DsbCD the growth inhibition was not observed. These results shows that the expression of folding proteins could be used to improve the cell growth and HRP production. (28 ref)

E.C. NUMBERS: 1.11.1.7
DESCRIPTORS: horseradish recombinant peroxidase prep., plasmid pTrc-OmpA-HRP expression, Escherichia coli, molecular **chaperone** , foldase, Dsb folding accessory protein, coexpressin effect plant Armoracia rusticana enzyme EC-1.11.1.7 bacterium fermentation (Vol.20, No.6)
SECTION: BIOCATALYSIS-Isolation and Characterization; GENETIC ENGINEERING AND FERMENTATION-Nucleic Acid Technology; GENETIC ENGINEERING AND

FERMENTATION-Fermentation (K1,A1,A2)

Improvement of productivity of active horseradish peroxidase **in Escherichia coli by coexpression of Dsb protein**

ABSTRACT: The effect of folding accessory proteins, molecular chaperones and foldases on the productivity of active **horseradish peroxidase** (HRP, EC-1.11.1.7) was studied in Escherichia coli JM109. Plasmid pTrc-OmpA...

DESCRIPTORS: horseradish recombinant peroxidase prep., plasmid pTrc-OmpA-HRP expression, Escherichia coli, molecular **chaperone** , foldase, Dsb folding accessory protein, coexpressin effect plant Armoracia rusticana enzyme EC-1.11.1...

6/9,K/66 (Item 1 from file: 370)

00510242 (THIS IS THE FULLTEXT)

Xyloglucan Fucosyltransferase, an Enzyme Involved in Plant Cell Wall Biosynthesis

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Abstract: Cell walls are crucial for development, signal transduction, and disease resistance in plants. Cell walls are made of cellulose, hemicelluloses, and pectins. Xyloglucan (XG), the principal load-bearing hemicellulose of dicotyledonous plants, has a terminal fucosyl residue. A 60-kilodalton fucosyltransferase (FTase) that adds this residue was purified from pea epicotyls. Peptide sequence information from the pea FTase allowed the cloning of a homologous gene, AtFT1, from Arabidopsis. Antibodies raised against recombinant AtFTase immunoprecipitate FTase enzyme activity from solubilized Arabidopsis membrane proteins, and AtFT1 expressed in mammalian COS cells results in the presence of XG FTase activity in these cells.

Text: In most multicellular organisms, cells are embedded in a complex extracellular matrix that keeps them together and influences the shape, development, and polarity of the cells they contact. Animal cells have such an extracellular matrix at their surface, but plants possess a distinct wall that encloses every cell. Many important differences between plants and animals with respect to nutrition, growth, reproduction, and defense mechanisms can be traced to the plant cell wall (B1) . Cell wall extensibility is a major determinant of plant growth (B2) . The

biosynthesis of plant cell walls is very tightly regulated. Although an individual plant cell may expand its volume by nearly 20,000 times, its cell wall must maintain a uniform thickness and structure to prevent hemorrhaging of the cell through local defects (B2) . However, despite extensive descriptions of the chemical and physical structure of the plant cell wall, very little is known about its biosynthesis. One gene encoding a cell wall-synthesizing enzyme, cellulose synthase, has been cloned (B3) .

The flexible primary walls of young plant cells are mainly composed of cellulose microfibrils and matrix polysaccharides. Matrix polysaccharides include hemicelluloses that bind tightly but noncovalently to cellulose microfibrils, cross-linking them into a complex network. The hemicellulose xyloglucan (XG) makes up approximately 20% of the total cell wall in dicot and nongraminaceous monocot plants and forms a load-bearing network by associating to the surfaces of surrounding cellulose microfibrils through hydrogen bonds (B4) (B5) . XG contains a (beta) -1,4-glucan backbone decorated with side chains of xylose alone; xylose and galactose; and xylose, galactose, and fucose. The presence or absence of the terminal fucose residue may have structural and biological significance. Some models suggest that the presence or absence of this fucose residue will determine whether the xyloglucan conformation is planar and thus better able to bind to cellulose (B6) , though contradicting evidence has been described (B7) . XG networks may be modified by XG endotransglycosylase (XET), an enzyme that cleaves and rejoins adjacent XG chains. A recombinant XET demonstrated different activity rates for fucosylated versus nonfucosylated XG oligosaccharide acceptors, indicating that the fucosylation state may affect XET modification of the cell wall (B8) . In addition, oligosaccharides consisting of an XG nonasaccharide prevent auxin-promoted elongation of pea stems if these oligosaccharides contain fucose but not if they lack fucose (B9) . Thus, it is possible that XG fragments act as signaling molecules in vivo.

Most matrix polysaccharides are branched molecules modified by various sugars. These modifications are important because they allow heterogeneity in the shape of matrix polysaccharides and in the patterns of cross-links, resulting in a dynamic and porous cell wall. These polysaccharide modifications occur via glycosyltransferase reactions, many of which occur in the Golgi complex (B10) . Attempts to clone plant glycosyltransferases using sequences derived from bacterial or mammalian transferases have been unsuccessful (B11) . This is not entirely unexpected, for although Golgi glycosyltransferases often have similar general structural features, they rarely share extensive sequence similarity (B12) .

The terminal fucosyl residue on XG side chains is added by a fucosyltransferase (FTase). We purified enough of this FTase from pea epicotyls to determine partial amino acid sequences from the enzyme. Microsomes were prepared from the pea epicotyls, carbonate-washed to enrich for membrane proteins (B13) , and solubilized with nonionic detergent such as Triton X-100. A specific assay for this enzyme was developed using tamarind or nasturtium seed storage XG, which lack fucosyl residues, as acceptor molecules and radiolabeled guanosine diphosphate (GDP)-fucose as a donor (B14) (B15) . GDP-agarose affinity chromatography, size exclusion chromatography, and anion exchange chromatography were used in conjunction with FTase activity assays to purify and detect the enzyme (Fig. 1) (B16) . It was possible to purify XG FTase 1400-fold after size exclusion chromatography, resulting in a total of 50 (mu) g of protein containing 70 nanokats (nKat) (nanomoles of substrate incorporated into the product per second) of XG FTase activity. To confirm that the purified pea protein synthesizes an a-1,2 fucose: galactose linkage, carbohydrate analysis was performed on the product resulting from in vitro fucosylation of tamarind

XG by purified FTase (Table 1) (B17) . Linkage analysis indicated that incubation of tamarind XG with purified FTase resulted in a decrease in the mole percentage of terminal galactose and the appearance of 2-galactose and terminal fucose, thus verifying the activity of the purified enzyme (Table 1). After biochemical purification and subsequent analysis, two polypeptides of approximately 65 and 60 kD in size were observed to copurify with XG FTase activity (Fig. 1). Limited peptide sequences were obtained from both proteins (B18) . The 65-kD peptide was identified as a homolog of BiP, a molecular **chaperone** usually localized to the endoplasmic reticulum. It remains unclear whether copurification of BiP with FTase represents an important interaction. Six peptides analyzed from the 60-kD protein were not significantly similar to proteins of known function in databases but did allow the identification of an Arabidopsis expressed sequence tag (EST), the sequence of which encoded four out of the six peptides, with amino acid identity ranging from 63 to 85% (B18) .

An analysis of this EST (number 191A6T7) indicated that it was not a full-length clone (B19) . The EST was used as a probe to screen an Arabidopsis cDNA library, and full-length cDNA clones were isolated (B19) . The cDNA clones contain a 1677-nucleotide open reading frame encoding a 63.5-kD protein and correspond to a region of the fully sequenced Arabidopsis bacterial artificial chromosome (BAC) T18E12 (B20) . The cDNA and the corresponding genomic clone have been designated AtFT1. Analysis of the BAC indicates that there may be a second glycosyltransferase approximately 300 base pairs downstream from AtFT1 that is 63% similar to AtFT1 at the amino acid level. In addition, two other Arabidopsis ESTs and three Arabidopsis genomic sequences that show significant similarity to AtFT1 have been observed in the databases (B21) . Thus, Arabidopsis may carry a family of FTases, each differentially regulated by such factors as environmental stress, tissue localization, or developmental stage, or specific to different acceptors.

To confirm the identity of AtFT1 as encoding a fucosyltransferase using XG as an acceptor, we prepared polyclonal antibodies directed against AtFT1 overexpressed in Eschicheria coli and used them to immunoprecipitate proteins from carbonate-washed, detergent-solubilized Arabidopsis proteins (B22) (B23) . The immunoprecipitated proteins were then assayed for XG FTase activity; 2.6-fold more FTase activity was correlated with pellets derived from immunoprecipitation reactions using immune antiserum rather than preimmune serum, thereby confirming that the Arabidopsis clone encodes a XG FTase (Fig. 2). In addition, a COS cell line expressing AtFT1 showed in vitro FTase activity that was 41 times higher than that of COS cells transformed with an empty vector (Fig. 2) (B24) . Taken together, these data indicate that AtFT1 is involved in XG biosynthesis.

Although AtFT1 has some structural characteristics common to other fucosyltransferases, it is quite divergent at the amino acid sequence level. Hydrophobicity plots predict that there may be an NH.inf(2)-terminal transmembrane signal anchor sequence. In vitro translation in the presence of canine pancreatic microsomes followed by carbonate washing of the products indicates that the AtFT1 translation product is a membrane protein (B25) . As with other glycosyltransferases, the COOH-terminal region is predicted to be largely hydrophilic. AtFT1 is not significantly similar to any other FTases from other organisms, although multiple sequence alignments have identified three motifs that appear to be conserved among all a1,2-FTases (B26) . One of these motifs, described previously, is found in all a1,2-and a1,6-FTases for which sequence data are known (B27) . Because these proteins have different acceptor molecules but share the same sugar nucleotide donor (GDP-fucose), it is possible that these regions are involved in GDP-fucose binding or conserved structural characteristics.

Some small regions of similarity are observed between AtFT1 and NodZ, a fucosyltransferase in Rhizobium involved in the synthesis of nodulation factors.

The unique nature of this FTase will allow its use as a tool for identifying other plant-specific glycosyltransferases. Hundreds to thousands of different genes (B28) are needed to synthesize the various polysaccharides that compose the cell wall. Substrate acceptors and assays remain unavailable for many of these enzymes. Identification of other carbohydrate transferases, perhaps by sequence similarity, could lead to tailored in vitro production of carbohydrates as well as an understanding of how the complex plant cell wall is biosynthesized.

Figure F1
Caption: Biochemical purification of XG FTase from pea. (A) Silver-stained SDS-PAGE gels showing protein profiles from (i) carbonate-washed detergent-solubilized microsomes from pea epicotyls (Sol. Microsomes) or the fractions containing peaks of FTase activity from (ii) a GDP-agarose affinity column (GDP-affinity) or (iii) a size exclusion column (Size Excl.) (B15) . Numbers at left indicate sizes in kilodaltons. (B) Top: Silver-stained SDS-PAGE gel showing the protein profile from several fractions of an anion exchange column. Numbers at left indicate sizes in kilodaltons. Bottom: Total XG FTase activity for each fraction of the anion exchange column eluate. Bars align with the corresponding SDS-PAGE profiles above.

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Begin Table : Columns 1 - 3 of 3

Caption:
Carbohydrate linkage analysis of tamarind XG before (tamarind XG) and after (fucosylated XG) incubation with purified pea FTase Reference B17 . Dashes indicate that no such linkage was detected.

Sugar residue	Tamarind XG (%)	Fucosylated XG (%)
4-glucose	16.4	17.5
4,6-glucose	37.0	31.5
Terminal xylose	19.0	13.5
2-xylose	15.0	14.3
Terminal galactose	12.6	5.5
2-galactose	-	9.0
Terminal fucose	-	8.7

End Table: Columns 1 - 3 of 3

Figure F2
Caption: Confirmation of AtFTase activity. (A) Polyclonal antibodies to AtFT1 recognize an approximately 63-kD polypeptide in solubilized membrane proteins of Arabidopsis. Top: Left two lanes, immunoblot; right two lanes, Coomassie blue staining of immunoblot membrane. In both cases, lane 1 is Arabidopsis carbonate-washed, detergent-solubilized membrane proteins, and lane 2 is antigen (50 ng). Bottom: Antibodies to FTase immunoprecipitate more XG-specific FTase activity than does an equal volume of preimmune serum. The FTase activity of precipitated pellets is shown. This is an example similar to results seen in seven different replicates. (B) Full-length AtFT1 expressed in a Cos cell line shows XG-specific FTase activity. Activity is shown in the presence (+XG) or absence (-XG) of

tamarind XG for untransformed Cos-7 cells, cells transformed with vector DNA (Cos-7 vector), cells transformed with vector containing AtFT1 (Cos-7 vector AtFT1), or solubilized pea Golgi vesicles (Pea Golgi). In graphs, error bars show +/-1 SD; if no error bars are visible, SDs are contained within the width of the plot element.

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15. Samples were incubated at room temperature for 20 min (for immunoprecipitation samples) or 30 min (for protein purification samples) with 25 mM Pipes-KOH (pH 6.2), tamarind XG (0.5 mg/ml), and GDP-[.sup(3)H]-fucose to a final concentration of 3.3 nM (573 GBq/mmol; NEN, Boston, MA). Most assays also contained 50 (mu) M nonradiolabeled GDP-fucose. Assays of immunoprecipitation samples also contained 5 mM MgCl.inf(2). Reactions were precipitated with 70% ethanol, and .sup(3)H incorporation was measured by scintillation counting. The amount of fucose incorporated into the product was used to calculate activity in nanokats. ;
16. Two-centimeter segments, excised just below the apical hook of etiolated Pisum sativum, cv Alaska, were collected and homogenized in 1.5 volumes of buffer [50 mM Hepes (pH 7.5); 1 mM EDTA (pH 8.0); 0.4 M sucrose; 1 mM dithiothreitol (DTT); 0.1 mM phenylmethylsulfonyl fluoride (PMSF); and 1 (mu) g each of aprotinin, leupeptin, and pepstatin per

millimeter]. The homogenate was filtered and centrifuged at 2,000g for 15 min, and the supernatant was centrifuged at 100,000g for 1 hour. The resulting pellets were washed and homogenized in the presence of 0.1 M Na.inf(2)CO.inf(3) to strip away peripheral membrane proteins (B13) . The suspension was centrifuged at 100,000g for 1 hour, and the resulting pellets were washed and resuspended in buffer [50 mM Pipes-KOH (pH 6.2), 20% glycerol, 1 mM EDTA, 1 mM DTT, 0.1 M PMSF, and 1 (mu) g each of aprotinin, leupeptin, and pepstatin per millimeter]. The suspension was homogenized, Triton X-100 was added to a final volume of 0.8%, and the sample was stirred for 1 to 2 hours to solubilize membrane proteins before centrifugation a final time at 100,000g for 1 hour. Supernatant was collected and saved. When Arabidopsis cell suspension culture was used as a tissue source, the procedure was identical except that the cells were lysed with a French pressure cell at 4000 psi. Pea carbonate-washed supernatants were pooled and separated on a GDP agarose affinity chromatography column, and GDP-binding proteins were eluted by means of excess free GDP. Protein levels were monitored by absorbance at 280 nm. The protein samples were desalted on a Sephadex G-25 column, concentrated, and further separated on a Phenomenex SEC 4000 size exclusion column. Some samples were further purified with a Poros QE or Resource Q anion exchange column and were subsequently separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). ;

17. Tamarind seed XG was fucosylated by purified pea FTase equal to 33 pKat total activity [assay conditions were as follows: tamarind XG (1 mg/ml), 1.5 mM GDP-fucose, and 50 mM Pipes-KOH (pH 6.2)]. The XG product was precipitated with ethanol, resuspended in water, reprecipitated, and sent to the Complex Carbohydrate Research Center (Athens, GA) for linkage analysis. An equal amount of tamarind XG was also submitted for linkage analysis. ;

18. Proteins in size exclusion column eluate fractions containing peak amounts of FTase activity were concentrated with a Millipore 4-ml 10-kD concentrator and separated by electrophoresis. After brief staining with Coomassie brilliant blue R250 and destaining, the separated proteins were excised, rinsed in 50% acetonitrile, stored at -80.Deg.C, and sent to Harvard Microchemistry (Cambridge, MA) for tryptic peptide sequencing. The following six peptide sequences were obtained: VFGFLGR, YLLHPTNNVWGLVVR, AVLITSLSSGYFEK, YYDAYLAK, LLGGLLADGFDEK, and ESILPDVNR (B29) . Using these peptides as a query, the Blastp program identified an Arabidopsis EST, 191A6T7, which encoded four out of six peptides. ;

19. Northern (RNA) blot analysis using the 858-nucleotide EST 191A6T7 as a probe detected an approximately 2-kb transcript, which indicates that the EST did not contain the full-length cDNA (R. M. Perrin, data not shown). 191A6T7 was used as a probe to screen the CD4-15 portion of a size-fractionated Arabidopsis cDNA library [J. J. Kieber, M. Rothenberg, G. Roman, K. A. Feldmann, J. R. Ecker, Cell 72, 427 (1993)] at high stringency. Two cDNA clones were isolated, the longest containing a 1768-base pair insert. Both lacked 13 nucleotides of the 3 (prime) UTR and the polyadenylated tail found in 191A6T7. There is an AATAAA consensus polyadenylation signal eight nucleotides from the 3 (prime) end of the library-derived clones. The sequence contains a 1677-nucleotide open reading frame that encodes a 63.5-kD protein. The translation product also contains a region near the NH.inf(2)-terminus that is similar to a fifth pea peptide (B18) . The nucleotide sequence of AtFT1 has been assigned the GenBank accession number AF154111. ;

20. AtFT1 is encoded within BAC T18E12, derived from chromosome II (nucleotides 41209 through 41503 and 41780 through 43252), which has been fully sequenced by the Arabidopsis Genome Initiative. A second open

reading frame has been predicted within T18E12 (nucleotides 43562 through 43748 and 43813 through 45215), which shows 63% similarity to AtFT1 at the deduced amino acid level. ;

21. R. M. Perrin, N. V. Raikhel, K. Keegstra, C. Wilkerson, unpublished results. ;

22. The portion of AtFT1 encoding amino acids 73 through 566 was amplified by polymerase chain reaction using appropriate primers and cloned into the pET28a expression vector (Novagen, Madison, WI). The resulting insoluble fusion protein was purified by washing of inclusion bodies four times with 1% Triton X-100, 50 mM Hepes-KOH (pH 7.6), and 10 mM MgCl.inf(2) and washing one time with 25 mM Hepes-KOH (pH 7.0) and 8 M urea. The pellet was resuspended in 6 M guanidine-HCl, and protein was precipitated from the supernatant with 10% trichloroacetic acid. The protein was emulsified with Titermax adjuvent (CytRx Corporation, Norcross, GA) and injected into a rabbit. For protein immunoblotting, 40 (mu) l of carbonate-washed solubilized protein from Arabidopsis and 50 ng of purified antigen were separated by SDS-PAGE and electroblotted. Antibodies to AtFT1 (dilution, 1:5000) were used for protein immunoblotting. **Horseradish peroxidase** -conjugated goat antibodies raised against rabbit antibodies were used as secondary antibodies. Signals were detected by the enhanced chemiluminescence method (Pierce, Rockford, IL). Membranes were stained with Coomassie blue to detect protein. ;

23. For immunoprecipitations, solid NaCl was added to carbonate-washed solubilized Arabidopsis protein to a final concentration of 200 mM. The Arabidopsis protein was precleared by incubation with 1:10 volume of a 50% slurry of protein A-Sepharose beads (Pharmacia) in buffer A [25 mM Pipes-KOH (pH 7.5), 50 mM NaCl, and 2 mM EDTA (pH 8.0)]. The resulting supernatants were incubated with 50 (mu) l of immune or preimmune antiserum to AtFT1 for 1 hour. A 1:5 volume of protein A-Sepharose slurry was added to precipitate the antigen-antibody complexes, and the samples were incubated for an additional 3 hours with rocking at 4.Deg.C. Samples were then centrifuged and washed five times in buffer A containing 1% Triton X-100 and two times in buffer A without detergent. The pellets were resuspended in buffer A to a final volume of 120 (mu) l and assayed for AtFTase activity as described above. ;

24. Cos-7 cells were grown on 100-mm plates in Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum. Cells were transfected with different plasmids using Lipofectamine reagent (Life Technologies) according to the manufacturer (prime) s instructions, using 9 (mu) g of DNA and 72 (mu) g of Lipofectamine. Cells were incubated for 24 hours in the medium containing DNA and Lipofectamine without fetal bovine serum. The medium was then changed to DMEM plus 10% fetal bovine serum and incubated for another 48 hours. The cells were scraped off the dish in 0.25 M sucrose, 10 mM tris-HCl (pH 7.5), and 0.4% CHAPS. XG-FTase activity was measured with 50 (mu) g of protein in the absence or presence of 100 (mu) g of tamarind XG. The incubation was carried out in a volume of 0.1 ml in the presence of 1 (mu) M GDP-fucose (93,000 disintegrations per minute), 10 mM MnCl.inf(2), 20 mM Hepes (pH 7.0), and 0.05% Triton X-100 at 25.Deg.C for 90 min. The reaction was halted by the addition of ethanol to a final concentration of 70%. Samples were incubated at 4.Deg.C and filtered through 1.5- (mu) m glass fiber filters. The filters were washed with 70% ethanol containing 1 mM EDTA. The filters were then dried and radioactivity was determined by liquid scintillation. A biological control using pea Golgi vesicles was carried out in parallel. ;

25. R. M. Perrin, K. Keegstra, N. V. Raikhel, data not shown. ;

26. Three motifs were found to be conserved among several a-1,2-fucosyltransferases, despite low overall homology. One ([IV]G[IV][HQ][VI]R..[DN]) has been described previously (B27) (square brackets indicate that either of the indicated amino acids was found at the indicated position; dots indicate that three or more different amino acids were found at the indicated position). In addition, a second motif (D[EK][MQ][FI]F[CR][EQ].DQ) and a third region (G[LF]G[ND][RC][IL].[TS][LI]A[SA].[FW][LR][YF]A.[LQ]T[DG]R..[LA].[VI][DE]) were conserved (B29) . ;
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29. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. ;
30. The authors acknowledge funding from the Department of Energy (grant DE-FG02-91ER20021), C. Wilkerson for assistance with computer analysis, and members of the Keegstra and Raikhel laboratories for helpful discussions.
Descriptors: Physics

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References and Notes:
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6/9,K/67 (Item 2 from file: 370)

00501653 (THIS IS THE FULLTEXT)
A Critical Role for Tapasin in the Assembly and Function of Multimeric MHC Class I-TAP Complexes
Ortmann, Bodo; Copeman, James; Lehner, Paul J.; Sadasivan, Bhanu; Herberg, Jethro A.; Grandea, Andeas G.; Riddell, Stanley R.; Tampe, Robert; Spies, Thomas; Trowsdale, John; Cresswell, Peter
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Abstract: Newly assembled major histocompatibility complex (MHC) class I molecules, together with the endoplasmic reticulum **chaperone** calreticulin, interact with the transporter associated with antigen processing (TAP) through a molecule called tapasin. The molecular cloning of tapasin revealed it to be a transmembrane glycoprotein encoded by an MHC-linked gene. It is a member of the immunoglobulin superfamily with a probable cytoplasmic endoplasmic reticulum retention signal. Up to four MHC class I-tapasin complexes were found to bind to each TAP molecule. Expression of tapasin in a negative mutant human cell line (220) restored class I-TAP association and normal class I cell surface expression. Tapasin expression also corrected the defective recognition of virus-infected 220 cells by class I-restricted cytotoxic T cells, establishing a critical functional role for tapasin in MHC class I-restricted antigen processing.

Text: Major histocompatibility complex molecules with specific bound peptides are the ligands for the antigen-specific receptors of T cells. Appropriate expression of MHC molecules with associated self peptides in the thymus and peripheral tissues is essential for the maintenance of T cell tolerance, and effective responses to infectious organisms require efficient assembly of both MHC class I and class II complexes with pathogen-derived peptides. Assembly of the MHC class I-peptide complex is initiated in the endoplasmic reticulum (ER) by the formation of MHC class I- (beta) .inf(2)-microglobulin ((beta) .inf(2)M) dimers and predominantly involves the chaperones calnexin and calreticulin (B1) (B2) (B3) (B4) (B5) . The peptides bound by MHC class I molecules are mainly generated by the proteasome-mediated cleavage of cytosolic proteins (B6) . Peptides are translocated into the ER by TAP, a specialized transporter that is a member of the adenosine triphosphate (ATP)-binding cassette family of transporters. It is composed oftwo homologous MHC-encoded subunits, TAP.1 and TAP.2 (B7) . Before peptide binding, calreticulin-associated class I molecules bind to TAP, an interaction mediated by tapasin (B4) . In the mutant cell line 220, which lacks tapasin expression, this interaction does not occur, and MHC class I assembly and subsequent cell surface expression is impaired (B4) (B8) (B9) . Somatic cell genetic evidence has suggested that either a gene regulating the expression of tapasin or the tapasin gene itself resides on the short arm of human chromosome 6 (B8) (B10) .

Translocation by TAP of an allele-specific, class I-binding peptide initiates dissociation of that class I molecule from the TAP complex (B1) (B2) . To investigate how translocation is coupled to peptide loading, we purified and characterized the human TAP complex, identified the different components, and determined their stoichiometry. We purified the class I-TAP complex from digitonin-solubilized membranes from the human Epstein-Barr virus (EBV)-transformed B cell line L001 using an affinity column containing monoclonal antibody (mAb) (148.3) to TAP1 (anti-TAP.1) (B11) . The L001 cell line was used because it expresses relatively large amounts of TAP and because its class I molecules associate stably with TAP. The material eluted at pH 3.5 is shown in Fig. 1 A. On the basis of previous data, bands were tentatively identified as TAP.2 (72 kD), TAP.1 (70 kD), calreticulin (60 kD), tapasin (48 kD), human lymphocyte antigen (HLA) class I heavy chain (44 kD), and (beta) .inf(2)M (12 kD). We also detected a band at 90 kD, which is the apparent size of calnexin on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). To confirm the identity of the components, we analyzed similar material purified from the cell line Swei. Calnexin, TAP.2, calreticulin, tapasin, and HLA class I heavy chain were positively

identified by immunoprecipitation after biotinylation of the purified material (B12) (Fig. 1B).

The approximate stoichiometry of the TAP complex purified from L001 cells and that purified from (beta) .inf(2)M-negative Daudi cells was calculated by excising bands from SDS-PAGE gels and subjecting each band to quantitative amino acid analysis. Two preparations from L001 cells were analyzed, one eluted from the affinity column at pH 3.5 and the other by competitive elution with the specific TAP.1 COOH-terminal peptide to which mAb 148.3 was raised (Table 1). This method eliminates the possibility that TAP.1-associated molecules might dissociate while leaving residual TAP.1 on the column. The ratio of TAP.1 to TAP.2 is about 1:1 as anticipated on the basis of previous characterization (B13) . In L001 cells the ratio of tapasin to HLA class I heavy chain is also about 1:1. This argues for a single class I binding site on the tapasin molecule. The ratio of tapasin to TAP dimer is considerably greater than 1:1 in both Daudi and L001 cells, and the data indicate that up to four tapasin-class I complexes are associated with a single TAP dimer, assuming that the purified material reflects a single complex of defined composition. The tapasin-TAP association is weak and detergent-dependent (B4) , necessitating the use of digitonin throughout the purification. Thus, the amount of tapasin relative to TAP after purification with an antibody to TAP is unlikely to be erroneously high. MHC class I molecules associate with TAP.1 in single-chain TAP.1-expressing cell lines (B1) (B14) , and we found that tapasin similarly associates with TAP.1 (B15) . Thus, the tapasin-class I complexes may all be bound to TAP.1, or a subset may be TAP.2-associated. Calreticulin association with class I molecules is maintained in the class I-TAP complex, although in one of the L001 preparations it was somewhat substoichiometric relative to class I and tapasin. This may reflect dissociation of calreticulin from the complexes after solubilization or variable association in vivo. Substoichiometric amounts of calnexin copurify with the complex, and preliminary evidence suggests that calnexin may be associated with a small number of assembling TAP complexes (B15) .

To determine the structure of tapasin, we separated the TAP-tapasin complexes from Daudi cells by SDS-PAGE, transferred them electrophoretically to Immobilon-P membrane, and excised the tapasin band. Edman degradation generated an unambiguous sequence from cycle 2 to cycle 31. We also identified residues 2 to 7 of a purified tryptic peptide. Degenerate polymerase chain reaction (PCR) primers were devised on the basis of the sequences and used to amplify fragments corresponding to the 5 (prime) end of the gene. We completed the sequencing using a modification of the vectorette, or "bubble," PCR approach (B16) adapted for use with cDNA.

Tapasin appears to be a type I membrane protein (Fig. 2 A). The mature protein has 428 amino acids with a single N-linked glycosylation site at position 233. The COOH-terminal sequence has lysine residues at positions -3, -4, and -5, consistent with known cytoplasmic ER retention signals that have lysine residues at -3 and -4 or -3 and -5 (B17) . The hydrophobic region likely to constitute the transmembrane domain (underlined in Fig. 2A) contains a basic residue, Lys.sup(408), which may be involved in intramembrane interactions with other proteins, for example, TAP. Such interactions occur between charged residues in the transmembrane domains of T cell receptor a and (beta) chains and CD3 components (B18) . Alternatively, Lys.sup(408) could be the COOH-terminal border of a 15-residue transmembrane domain beginning at Ser.sup(393). Hydrophobic sequences of comparable length can function as transmembrane sequences in recombinant membrane proteins (B19) . Of the 392 lumenal residues of the mature protein, 50 are prolines. They are distributed throughout the

sequence with no evidence of periodicity.

The tapasin polypeptide sequence was analyzed by Smith-Waterman database searching and by threading onto known protein structures (B20) , and by comparison with known immuno-globulin (Ig) sequences (B21) . All three methods indicated that tapasin is a member of the Ig superfamily. A sequence incorporating two cysteine residues (Cys.sup(295) and Cys.sup(362)) exhibited significant homology to members of the Ig C1-SET constant region superfamily (Fig. 2B). Many of the key amino acids characteristic of the C1-SET are present, but there are 67 amino acids between the two cysteines in tapasin, in contrast to the 55 to 60 residues in most C domains (B21) . The increased span may be due to an extended loop at residues 317 to 323, corresponding to an insertion between the C and D (beta) strands of the C1-SET structure. V-SET folds contain a large expansion at this position. An additional region with weak Ig C1-SET homology runs from residues 51 to 153, but the second Cys residue has not been conserved (B15) . The 118 residues (154 to 271) between these two putative Ig domains do not show any obvious Ig homology.

To map the gene encoding tapasin, we used a PCR probe that gave a positive signal on two human chromosome fragment-rodent somatic cell hybrids whose only common human chromosome band includes the MHC (Fig. 3 A). This is consistent with the location of the tapasin coding gene rather than a regulatory gene being on chromosome arm 6p (B8) (B10) . To refine the map position, we used a tapasin cDNA probe (base pairs 1 through 796) to screen gridded libraries of PAC clones (United Kingdom Human Genome Mapping Project resource center). This yielded a single clone, PAC 36A2, which we labeled with biotin and used for fluorescence in situ hybridization (FISH) analysis on 40 metaphase spreads from phytohemagglutin-stimulated normal human lymphocytes (B22) . A consistent signal was observed at band 6p21.3 on both copies of chromosome 6 (Fig. 3B). This chromosomal band includes the MHC. The result indicates that the tapasin gene is within ~10.sup(6) bp of the MHC.

The mutant cell line 220 is defective in MHC class I assembly and lacks tapasin expression (B4) (B8) (B9) . To determine whether introducing tapasin could restore a normal phenotype, we transfected tapasin cDNA into 220 cells expressing HLA-A1 or HLA-B8 (B8) . Immunoprecipitation with anti-TAP or anti- (beta) .inf(2)M showed that tapasin restored the association of HLA-B8 with TAP in 220-B8 cells (Fig. 4). Restoration of a functional TAP-tapasin-class I complex also resulted in an ~10-fold increase in class I surface expression (Fig. 5). The restored surface expression is similar to that seen in normal HLA-B8- or HLA-A1-positive B cell lines (B15) . To address the functional importance of tapasin, we infected 220-B8, 220-A1, and their tapasin-expressing derivatives with either influenza virus A/PR8/(HIN1) or a vaccinia virus recombinant expressing cytomegalovirus (CMV) pp65 protein and tested for their susceptibility to lysis by HLA-B8-restricted, influenza nucleoprotein-specific, or HLA-A1-restricted CMV pp65-specific, cytotoxic T cell lines (CTLs), respectively. Tapasin expression restored CTL sensitivity to virus-infected 220-B8 and 220-A1 cells (Fig. 5, C and D), indicating that tapasin plays an essential role in MHC class I function.

MHC class I molecules are more selective than TAP, in that they bind restricted sets of peptides, whereas TAP is highly promiscuous in the peptides it translocates (B23) . Thus, having multiple class I molecules associated with a single TAP dimer may increase the probability that an individual translocated peptide binds successfully. Certain HLA class I alleles have been found to associate poorly with TAP (B24) , which may reflect a weak interaction with tapasin. However, absence of association in detergent solution may not reflect the situation in vivo where the

components of the complex remain membrane-bound. The extent to which the antigen processing and presentation function of these weakly associated class I alleles is affected by the absence of tapasin remains to be determined.

It remains unclear precisely how tapasin, TAP, and calreticulin combine to facilitate MHC class I peptide loading. Cells that lack TAP but express tapasin form calreticulin-class I-tapasin complexes in the ER (B4) , and HLA-A2 molecules in such cells are quite efficiently assembled and transported, binding a subset of signal sequence-derived peptides (B25) . Thus TAP, although it is the primary source of peptides, is not absolutely required for peptide loading of class I molecules. HLA-B8 molecules expressed in 220 cells are degraded more rapidly than when expressed in TAP-negative 174 cells (B10) . Therefore, one role of tapasin may be to stabilize class I molecules in the absence of associated peptides, similar to the way HLA-DM association stabilizes empty MHC class II molecules (B26) . Tapasin could also function like the invariant chain does in the class II system, preventing premature nonspecific interactions with unfolded proteins in the ER (B27) . The interaction with TAP may simply provide a means to ensure proximity to the source of translocated peptides, with no additional mechanistic role in peptide loading. These possibilities remain to be evaluated.

Figure F1
Caption: Components of the MHC class I-TAP complex. (A) The TAP complex was affinity-purified from the cell line L001 by elution at pH 3.5 from an anti-TAP.1 (B13) affinity column, ethanol precipitated, subjected to SDS-PAGE, and stained with Coomassie blue. Molecular size markers in kilodaltons are on the left. The components of the complex are identified on the right. Class I HC, HLA class I heavy chain. (B) The TAP complex similarly purified from the cell line Swei, but eluted with the specific COOH-terminal peptide of TAP.1, was biotinylated and denatured (B12) , and individual components immunoprecipitated with rabbit antisera to tapasin (B4) , calnexin (B28) , or calreticulin (Affinity Bioreagents, Neshantic Station, New Jersey), or the mAbs 435.3 (anti-TAP.2) (B29) or 3B.10.7 (anti-class I heavy chain) (B30) . After SDS-PAGE and electrophoretic transfer to an Immobilon-P membrane, the components were detected by **horseradish peroxidase** (HRP)-conjugated avidin and a chemiluminescent substrate.

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Begin Table : Columns 1 - 7 of 7

Caption:
Composition of the purified TAP complex.

Cell line	Daudi		L001		L001	
	Nano-moles	Ratio	Nano-moles	Ratio	Nano-moles	Ratio
Calnexin	ND	-	0.0086	0.74	0.0089	0.86
TAP.1	0.0126	1.17	0.0120	1.03	ND	-
TAP.2	0.0108	1	0.0116	1	0.0104	1
Calreticulin	0	-	0.0229	1.97	0.0362	3.48
Tapasin	0.0395	3.66	0.0489	4.22	0.0369	3.55
HLA class I HC	0	-	0.0469	4.04	0.0406	3.90

Footnote:
Elution at pH 3.5.

Footnote:
Elution with specific peptide.
Footnote:
ND, not done.
Footnote:
No visible band.
End Table: Columns 1 - 7 of 7

Figure F2
Caption: (A) Deduced amino acid sequence (B31) of tapasin. The signal sequence (residues -20 to -1) is italicized, and proline residues are in bold type. Lysine residues 408, within the putative transmembrane region (underlined), and 424, 425, and 426, constituting a putative ER retention signal, are bold and italicized. The asterisk (Asn.sup(233)) indicates the putative site of N-linked glycosylation. The cDNA sequence is available through GenBank (accession number: BankIt 122101 AF009510). (B) Tapasin is a member of the Ig superfamily. Comparison of residues 272 to 382 of tapasin (Tap.) to some other members of the Ig superfamily that contain a C1-SET domain. Numbering is from the NH.inf(2)-terminus of the mature protein. Residues identical to those in tapasin are shaded in black; similar residues are shaded in gray. Gaps are indicated by a dot. H., HLA. These have been introduced to maximize homology with the Ig-C1 fold.
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Figure F3
Caption: Location of the tapasin gene in the MHC region. (A) The somatic cell hybrids MCP-6 and 56-47 both contain chromosome 6 fragments whose overlap includes the MHC (B32) . The breakpoint on MCP-6 maps between D6S105 and D6S276, just telomeric of the class I region of the MHC (B15) . In cell 56-47, the chromosome extends from the end of chromosome 6, p arm, to centromeric of HLA-DP, as determined by PCR, but its break point has been determined cytogenetically. The PCR primers were TTTAGATCCGGCAGTGAC and TCCTGAGTGTAGAGAAGGAAG, yielding a product of 157 bp. Lane 4 contains mouse genomic DNA. (B) The fluorescent signal of labeled PAC 36A2 DNA containing the tapasin gene is shown on the G-banded chromosome and corresponding idiogram, at band 6p21.3.
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Figure F4
Caption: Tapasin in 220-B8 restores assembly of the TAP-class I complex. 220-B8 cells were transfected with tapasin cDNA subcloned in the pMCFR-PAC vector (B33) and positive clones identified by immunoblot with the R.gp48N (anti-tapasin) antiserum (B4) . Control (A and B) and tapasin-transfected (C and D) 220-B8 cells were labeled with [.sup(35)S]methionine and [.sup(35)S]cysteine for 20 min, and 1% digitonin extracts were immunoprecipitated with anti- (beta) .inf(2)M [(A) and (C)] and R.RING.4C (anti-TAP.1) (B4) [(B) and (D)] antisera and separated by two-dimensional (2D) electrophoresis as described (B4) . Arrowheads to the left of each panel represent class I heavy chain, those to the right represent tapasin, and vertical arrowheads represent (beta) .inf(2)M. To confirm the identity of the bands, digitonin extracts from tapasin-transfected 220-B8 cells were immunoprecipitated with R.RING.4C, separated by 2D electrophoresis, and following transfer to Immobilon-P membranes probed with R.gp48N (E). Simultaneous detection of class I heavy chains with mAb 3B10.7 (F) confirmed the relative position of the tapasin spot.
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Figure F5
Caption: Tapasin transfection restores MHC class I surface expression and CTL lysis of 220-B8 and 220-A1 cells. Control and tapasin-transfected 220-B8 (A) and 220-A1 cells (B) (B34) were analyzed for class I surface expression by flow cytometry by using the mAbs w6/32 (A) and GS142.1 (B) and fluorescein-conjugated rabbit antibody to mouse IgG. The control was a nonspecific isotype-matched mAb. For each series of transfectants, several clones showed a similar increase in surface amounts of MHC class I. (C) Influenza A-specific, HLA-B8/NP380-88 restricted CTLs were generated from peripheral blood mononuclear cells (B35) and assayed in a standard .sup(51)Cr release assay on tapasin-(up triangle and (square-solid)) and control-transfected ((open-circle)), influenza A-infected (up triangle and (open-circle)) 220-B8 target cells. (D) Tapasin-(up triangle) and control-transfected ((triangle-solid)) 220-A1 cells were infected with recombinant vaccinia-CMVpp65 for the indicated periods of time, and cytotoxicity was assayed with the CMVpp65-specific, HLA-A1-restricted CTL clone DLS13B7 (B36) at a saturating effector to target ratio in a standard 5-hour .sup(51)Cr release assay.

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blue (0.25%). The bands were excised and subjected to amino acid analysis by the Keck Foundation Biotechnology Resource Laboratory, Yale University. In combination with the known molecular weights of the proteins (exclusive of carbohydrate), these data were used to calculate the amount of each component. Glycine, cysteine, and tryptophan were excluded from the calculations of protein amounts. For NH.inf(2)-terminal sequencing, the proteins were electrophoretically transferred to Immobilon-P membrane (Millipore), the membrane stained with Coomassie blue, and the tapasin band excised. NH.inf(2)-terminal sequencing was performed by the Keck Foundation Biotechnology Research Foundation, Yale University. ;

12. TAP complexes isolated from Swei cells were dialyzed extensively against 10 mM bicine, 150 mM NaCl, pH 8.2, and biotinylated by adding NHS-LC-biotin (Pierce) to a final concentration of 200 (mu) M and incubating for 30 min at 4.Deg.C. Biotinylation was terminated by adding glycine to a final concentration of 10 mM. The biotinylated proteins were denatured by adding SDS (2%) and dithiothreitol (2 mM) and heating the preparation to 100.Deg.C for 5 min. For immunoprecipitation, the denatured proteins were diluted 10-fold with 1% Triton X-100 in TBS containing 10 mM iodoacetamide, incubated for 30 min at 25.Deg.C, and then precipitated with the appropriate antibodies and protein A- or protein G-Sepharose (Pharmacia) before being subjected to SDS-PAGE. Biotinylated proteins were detected with avidin-HRP and a chemiluminescent substrate (ECL, Amersham). ;

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16. From the sequence of residues 2 through 31 obtained by NH.inf(2)-terminal amino acid sequencing, and from a sequence from a tryptic peptide that proved to be residues 77 through 83 (Fig. 2A), oligonucleotide primers were designed according to preferred human codon usage, but they were degenerate where necessary in the last six bases. RNA was extracted from Swei cells with TRIZOL (Gibco-BRL) and reverse transcriptase-PCR performed. Products were gel purified and cloned into pCR2.1 (Invitrogen). We generated the cycle sequencing template by PCR using resuspended colonies of clones as the template. Sequencing was performed by the Keck facility (Howard Hughes Medical Institute, Yale Medical School). The remaining sequence was obtained from a walking approach by vectorette PCR [P. M. Sharp et al., Nucleic Acids Res. 16, 8207 (1988)]. Polyadenylated RNA was purified with Oligotex (Qiagen), and the cDNA prepared (Gibco-BRL). Restriction digestions of 500 ng of cDNA were performed with Alu I, Hae III, Bsa AI, Hinc II, Msl I, Eco RV with Xmn I, and Pvu II with Stu I, and the vectorettes were ligated. Vectorette PCR was performed with a tapasin-and a vectorette-specific primer, with the above cDNA digestions as separate templates. PCR products were cloned and sequenced as above. For each tapasin-specific primer, a number of products from different cDNA digestions were sequenced in both directions. New tapasin-specific primers were designed to the sequence obtained. Alignment of the multiple overlapping sequences allowed the derivation of a consensus sequence. We verified this by amplifying the full tapasin coding region from a separate synthesis of intact Swei cDNA using an enzyme with proofreading capability (Pfu DNA polymerase; Stratagene). Products were cloned into pCR2.1 and the clones sequenced in both directions. These sequences corresponded to the consensus sequence from vectorette PCR. Details of PCR reactions are

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37. We thank P. Freemont for help with the homology analysis, members of D. Sheer's laboratory for FISH analysis, J. Yewdell for supplying influenza virus, T. Novak for the pMCFR-PAC vector, K. Stone and the Keck Foundation Biotechnology Resource Laboratory at Yale University for their contributions, and N. Dometios for preparation of the manuscript. B.O. was supported by an AIDS-Stipendium of the German Cancer Research Center and P.J.L. by the Wellcome Trust. The work described was supported by the Howard Hughes Medical Institute and by a grant (AI30581) from the NIH (T.S.).
Descriptors: Immunology

(THIS IS THE FULLTEXT)

Abstract: Newly assembled major histocompatibility complex (MHC) class I molecules, together with the endoplasmic reticulum **chaperone** calreticulin, interact with the transporter associated with antigen processing (TAP) through a molecule called tapasin...

...Text: SDS-PAGE and electrophoretic transfer to an Immobilon-P membrane, the components were detected by **horseradish peroxidase** (HRP)-conjugated avidin and a chemiluminescent substrate...

6/9,K/68 (Item 3 from file: 370)
DIALOG(R)File 370:Science

00500873 (THIS IS THE FULLTEXT)
From Peptide Precursors to Oxazole and Thiazole-Containing Peptide Antibiotics: Microcin B17 Synthase
Li, Yue-Ming; Milne, Jill C.; Madison, Lara L.; Kolter, Roberto; Walsh, Christopher T.
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Abstract: Esherichia coli microcin B17 is a posttranslationally modified peptide that inhibits bacterial DNA gyrase. It contains four oxazole and four thiazole rings and is representative of a broad class of pharmaceutically important natural products with five-membered heterocycles derived from peptide precursors. An in vitro assay was developed to detect

heterocycle formation, and an enzyme complex, microcin B17 synthase, was purified and found to contain three proteins, McbB, McbC, and McbD, that convert 14 residues into the eight mono-and bisheterocyclic moieties in vitro that confer antibiotic activity on mature microcin B17. These enzymatic reactions alter the peptide backbone connectivity. The propeptide region of premicrocin is the major recognition determinant for binding and downstream heterocycle formation by microcin B17 synthase. A general pathway for the enzymatic biosynthesis of these heterocycles is formulated.

Text: A growing number of peptide-based natural products have been found to contain thiazole and oxazole heterocyclic rings and exhibit significant antifungal, antibiotic, antitumor, and antiviral biological activities (B1) . The five molecules depicted in Fig. 1, bleomycin A.inf(2), thiangazole, patellamide A, pristinamycin II.inf(A), and thiostrepton, exemplify the patterns of such heterocycle occurrence in molecules of therapeutic interest. The antitumor antibiotic bleomycin uses the bithiazole moiety to intercalate into DNA (B2) . The tandem four-ring structure of thiangazole including the (beta) -methyloxazoline provides potent antiviral activity against human immunodeficiency virus (B3) . Patellamides, cyclic octapeptides of marine origin, have antitumor properties (B4) . Thiostrepton, a protein synthesis inhibitor with four thiazole and one thiazoline ring is a signature secondary metabolite of Streptomycetes (B5) . Derivatives of pristinamycin (for example, RP59500) are currently in advanced clinical testing for combating vancomycin-resistant Gram-positive bacterial infections (B6) . The heterocyclic rings are likely to arise by cyclization of peptide precursors; for example, a -Gly-Cys-dipeptide sequence converted to an aminomethylthiazoline, then modified to the aminomethylthiazole, and analogously, -Gly-Ser-converted to aminomethyloxazoline and modified to aminomethyloxazole.

Until recently little was known about the molecular genetics or the enzymology of thiazole and oxazole ring biogenesis. The E. coli peptide antibiotic microcin B17 (MccB17), which represents a class of DNA gyrase inhibitors (B7) distinct from quinolone or coumarin drugs, contains four thiazoles and four oxazoles fashioned from six glycines, four cysteines, and four serines present in pre-MccB17 (Fig. 2) (B8) . These initial studies of MccB17 have provided both the genetic and biochemical opportunity to begin decoding the molecular logic for the construction of both isolated oxazole and thiazole rings and the 4,2-bisheterocycles because the tripeptide sequences Gly.sup(39)Ser.sup(40)Cys.sup(41) and Gly.sup(54)Cys.sup(55)Ser.sup(56) in pre-MccB17 yield a 4,2-linked oxazole-thiazole and a 4,2-thiazole-oxazole, respectively. Analogous to bleomycin, each of the bisheterocyles could be intercalation moieties involved in the DNA gyrase inhibition that leads to accumulation of the cleaved DNA intermediate (B7) .

The E. coli MccB17 operon has been characterized by genetic analysis and provisional roles assigned to seven open reading frames, mcbA, -B, -C, -D, -E, -F, and -G. The gene mcbA encodes the 69-amino acid (aa) pre-MccB17 polypeptide. Three genes, mcbB, -C, and -D, are required for the conversion of nascent pre-MccB17 to pro-MccB17 (Fig. 2) (B9) (B13) . The first 26 amino acids of pro-MccB17 are removed proteolytically to yield the active antibiotic (B11) (B14) , MccB17 (residues 27 through 69), that is transported out of the producing E. coli cell by McbE and McbF (B12) . MccB17 is then taken up by susceptible cells where gyrase is the killing target (B7) . A seventh gene, mcbG, provides immunity to the MccB17-producing strain (B12) .

Purification and characterization of active MccB17 synthase complex

required the availability of substrates and a specific assay for detection of cyclized products from acyclic peptide precursors. Prior pulse-chase studies on the expression and lifetime of pre-MccB17 revealed very rapid degradation (B15) , not unanticipated given the high glycine content including 11 out of 12 residues at positions 28 through 39 in the McbA nascent product (Fig. 2). We prepared three substrates (Fig. 3A): (i) the first 65 codons of mcbA were fused to (beta) -galactosidase, creating a fusion protein of 102 kD, McbA- (beta) -Gal, which has been useful for both in vitro and in vivo studies (B15) ; (ii) an NH.inf(2)-terminal hexa-histidine tag was fused in frame to mcbA, yielding His.inf(6)-McbA (B16) , which could be overproduced and affinity purified from E. coli cell extracts by Ni.sup(2+)-chelate chromatography; and (iii) a fragment containing the first 46 residues of McbA [McbA(1-46)] was synthesized, which included the putative 26-aa propeptide or leader sequence and the Gly.sup(39)Ser.sup(40)Cys.sup(41) tripeptide sequence that is a locus for bisheterocycle formation in MccB17 posttranslational maturation.

To assay these potential substrates we used a polyclonal antibody that recognizes mature MccB17 and pro-MccB17, but not pre-MccB17 (Fig. 2) (B14) , which we reasoned to indicate that this antibody recognized one or more heterocycles as determinants, which was later validated. We further determined that this antibody recognizes the bithiazole fragment used in the synthesis of bleomycin when it is conjugated to bovine serum albumin (BSA) (B17) . When each of the three substrates was incubated with an E. coli extract containing McbB, -C, and -D, a linear signal was detected by protein immunoblot analysis with antibodies to MccB17 (anti-MccB17). Data for the McbA- (beta) -Gal substrate is shown in Fig. 3B. The enzymatic activity monitored by immunoblot required the presence of each of the three proteins McbB, McbC, and McbD, as assessed by an in vitro assay with knockout strains (mcbB.sup(-), mcbC.sup(-), and mcbD.sup(-)) (B18) (B19) .

Purification of MccB17 synthase from the antibiotic-producing strain ZK4(pPY113) (B14) (B20) resulted in a 59-fold increase in specific activity (B21) . Starting from 3.6 g of E. coli soluble protein, we obtained 9.5 mg of the purified protein with an overall yield of 16%. Purified MccB17 synthase has a Michaelis constant K.inf(m) of 2.3 (mu) M for McbA(1-46) and an estimated catalysis constant k.inf(cat) of 0.2 min.sup(-1), on the basis of formation of both heterocyclic rings (B22) .

SDS-PAGE analysis of purified MccB17 synthase revealed three major protein bands at apparent molecular sizes of 68, 45, and 31 kD (Fig. 4A). NH.inf(2)-terminal sequencing and protein immunoblot analysis demonstrated that the 31-kD band contained two proteins, McbB (33 kD) and McbC (31 kD), and the 45-kD band was McbD. The NH.inf(2)-terminal sequence of the purified proteins showed that (i) McbB was translated from the second potential start site rather than the first one (B13) , (ii) the first Met was removed in McbC, and (iii) the open reading frames for mcbC and mcbD overlap by 20 bases (B13) . The fourth protein, at ~68 kD, had the sequence of HtpG, an E. coli member of the Hsp90 heat shock protein family (B23) .

In a parallel set of experiments we separately overproduced McbB, His.inf(6)-McbC, and His.inf(6)-McbD (B24) , purified each to homogeneity, and raised monospecific polyclonal antibodies with no cross-reactivity to each other. We used these three antibodies to confirm that McbB, -C, and -D make up a MccB17 synthase complex by immunoprecipitating them from crude extracts of E. coli ZK4(pPY113). McbB is co-precipitated with McbC and McbD by anti-McbC or anti-McbD (Fig. 4B). McbC is co-precipitated by anti-McbB and anti-McbD. McbD is not well resolved from the immunoglobulin G bands in such immunoprecipitates. We do not yet know the stoichiometry of McbB, -C, and -D in the active MccB17 synthase complex, although enzyme activity migrates on a size-exclusion column with an apparent molecular size of ~100

kD, minimally consistent with unitary stoichiometry of McbB (33 kD), McbC (31 kD), and McbD (45 kD).

Given that the activity assay to this point relied on formation of antibody-positive material, we sought to define the nature of the enzymatic modification more precisely. We turned to both the His.inf(6)-McbA construct with eight potential sites for heterocycle formation and McbA(1-46), which contains two modifiable residues Ser.sup(40) and Cys.sup(41), but no others. Both peptides were substrates in the anti-MccB17 protein immunoblot assays as described above, but the question arose as to how many modifications were being introduced in vitro by purified MccB17 synthase. Purified MccB17 synthase was incubated with McbA(1-46) for 45 min, then subjected to high-performance liquid chromatography (HPLC). The product of the McbA(1-46) peak was recognized by anti-MccB17. Mass spectrometric (MS) analysis revealed that the mass of the starting material was 4160.4 [calculated mass for McbA(1-46), 4161.8], and the mass of the antibody-positive material was 4119.8 (Fig. 5). The 4120 peak was absent from the controls at zero time or if MccB17 synthase was omitted from the incubation mix. Conversion of the Gly.sup(39)Ser.sup(40)Cys.sup(41) tripeptide moiety into the 4,2-oxazole-thiazole resulted in the loss of 40 mass units for a calculated mass of 4121.8, very close to the observed 4119.8 (Fig. 5). Two points are thus established. First, anti-MccB17 recognizes heterocycle-containing product, and second, the tandem bisheterocycle is made from McbA(1-46), with no accumulation of either the monocyclic thiazole or oxazole intermediate (calculated mass of 4142) at the time point examined. The His.inf(6)-McbA construct has all the eight possible sites of heterocycle modification for a total decrease in mass of 8 x 20 = 160 mass units if all sites are recognized. In the event, incubation of His.inf(6)-McbA with purified enzyme complex, followed by HPLC and initial localization of the product by protein immunoblot analysis, gave fractions with mass of 7888.10 (calculated mass of fully modified product = 7886.40), whereas the starting material (His.inf(6)-McbA) in the absence of enzyme or in the time zero sample gave 8058.30 (calculated = 8046.4), for a loss of 170 mass units. These data demonstrate that purified MccB17 synthase is able to convert four serines and four cysteines in premicrocin (McbA) to the full complement of heterocycles found in the antibiotic.

Given the effective processing of McbA(1-46) by purified MccB17 synthase, we prepared the fragment McbA(27-69) (Fig. 3A) that contains the 43 aa that end up in the mature, posttranslationally processed MccB17 antibiotic. In contrast to the McbA(1-46) substrate behavior, we could detect no enzymatic conversion of McbA(27-69) over a concentration range of 2 to 140 (mu) M, under conditions where we could easily have detected 5% of the signal produced with McbA(1-46). We estimate by k.inf(cat)/K.inf(m) (catalytic efficiency) that the 43-aa fragment, McbA(27-69), is at least 500-to 1000-fold less efficient than McbA(1-46). Next, we assessed the ability of McbA(27-69) and also a peptide composed of residues 27 through 46 [McbA(27-46)] to block processing of McbA(1-46) and observed no inhibition at 80 (mu) M McbA(27-69) or McbA(27-46). In contrast, the 26-aa propeptide, McbA(1-26), inhibited modification of McbA(1-46) with an IC.inf(50) (median inhibitory concentration) of 2 (mu) M, essentially the same as the K.inf(m) for McbA(1-46) (B25) . Taken together, these results indicate that most if not all of the recognition of substrate by MccB17 synthase is provided by the leader or propeptide region. This is reminiscent of the eukaryotic vitamin K-dependent carboxylase posttranslational modification of the first 10 to 12 glutamyl residues to (gamma) -carboxyglutamates in such zymogens as factor IX, factor X, and prothrombin (B26) . In these proteins an 18-residue propeptide region, the

(gamma) -carboxylase recognition sequence, also provides a 1000-fold increase in affinity for downstream modification of peptide substrates. Recent data concerning the nisin family of lanthionine polypeptide antibiotics also indicate interaction of the propeptide region with a multimeric lanthionine synthetase complex (B27) . By analogy, one or more of the McbB, -C, or -D subunits may recognize the propeptide(1-26) region before downstream heterocycle formation (B28) .

An initial survey of potential cofactor requirements for MccB17 synthase-mediated conversion of Ser and Cys residues into oxazole and thiazole rings was conducted with the McbA- (beta) -Gal fusion protein as a substrate. One requirement, apparent from the protein immunoblot assay, was adenosine triphosphate (ATP), which has a K.inf(m) of 89 (mu) M. Guanosine triphosphate (GTP) has a K.inf(m) of 52 (mu) M, but with one-third the k.inf(cat). Adenosine-5 (prime) -((gamma) -thio)triphosphate (ATP- (gamma) -S) could also serve as a substrate, but at a K.inf(m) (141 (mu) M) 1.6 times that of ATP and with a 76-fold lower relative k.inf(cat), for a 122/1 ratio of ATP/ATP- (gamma) -S by k.inf(cat)/K.inf(m) catalytic efficiency criterion. The ATP analog adenosine diphosphate-CH.inf(2)-PO.inf(3.sup(-)) (AMP-PCP) is a competitive inhibitor, with an inhibitory constant K.inf(i) of 2.3 (mu) M. Analysis with [(gamma) -.sup(32)P]ATP or [a-.sup(32)P]ATP revealed only adenosine diphosphate and inorganic phosphate (P.inf(i)) as products. As yet, we cannot report ATP and heterocycle stoichiometry because of some contaminant peptide-independent adenosine triphosphatase activity.

For each serine or cysteine converted to a heteroaromatic five-membered ring product oxazole or thiazole, two electrons are removed. This redox stoichiometry requirement prompted us to look both for an external electron acceptor and for some redox-active cofactor in the MccB17 synthase complex. One possibility was that nicotinamide adenine dinucleotide (NAD.sup(+)) or NAD phosphate (NADP.sup(+)) might serve as an electron acceptor in the step converting thiazolines to thiazoles or oxazolines to oxazoles (Fig. 6A). However, addition of NAD.sup(+) or NADP.sup(+) to the reaction mixture with purified MccB17 synthase had no effect on the rate of product formation (B18) . The most likely terminal electron acceptor then was O.inf(2). Incubations conducted on degassed assay components yielded a reduction in the rate of protein immunoblot-positive product by 2.1-fold for McbA(1-46) under conditions where a glucose-glucose oxidase test system had initial velocity reduced three-to fivefold (B29) . MS analysis on the McbA(1-46) assay revealed no discernible difference between the product produced at lowered O.inf(2) pressure as compared with normal assay conditions. More stringent anaerobiosis will be required to determine a K.inf(m) for O.inf(2) and to test whether any oxazoline and thiazoline species form under these conditions.

Although sequence analysis of McbB, -C, and -D failed to detect any signature motifs for cofactors (including for ATP or GTP), it seemed likely that one or more of McbB, -C, or -D should contain a bound redox-active metal or conjugated organic coenzyme to facilitate two-electron oxidation of the proposed thiazoline and oxazoline intermediates (Fig. 6A). Indeed, overproduced and purified McbC was yellow and contained a stoichiometric amount of tightly but noncovalently bound FMN (B18) (B30) .

The detection and purification of MccB17 synthase opens the door for future deconvolution of several aspects of how five-membered ring heterocycles are fashioned enzymically from acyclic peptide precursors, including propeptide recognition, regioselectivity, processivity, minimal size, and number of heterocycles for DNA gyrase inhibition and antibiotic activity. At this point, given our results that reveal elements of the

molecular logic for serine-to-oxazole and cysteine-to-thiazole conversions, the mechanistic scheme proposed in Fig. 6A delineates how the Gly.sup(34)Ser.sup(40)Cys.sup(41) tripeptide moiety of MccB17, and specifically in McbA(1–46), is converted to the aminomethyl bisheterocyclic 4,2-oxazole-thiazole, a likely DNA intercalator for subsequent interaction with a DNA-DNA gyrase complex. In both serine-to-oxazole and cysteine-to-thiazole transformation for MccB17, and most likely for all such peptide-derived natural products (for example, Fig. 1), we propose a three-step sequence of cyclization, net dehydration, and subsequent two-electron dehydrogenation.

It is tempting, given three proteins, McbB, -C, and -D, as necessary constituents in MccB17 synthase action, to assign each protein one of these three enzymatic functions, but that would be premature. The cyclization is itself remarkable in likely geometric requirements, and it is reasonable that Gly–Ser and Gly–Cys sequences are favored for cyclization both for lack of steric bulk in the a-CH.inf(2) group of the Gly residue and the Ramachandran angles populated by Gly-X dipeptides. The initial cyclic adduct is likely to be disfavored compared with the acyclic ground state unless the oxyanion formed from the Gly–Ser carbonyl oxygen can be diverted. An obvious route would be enzyme-assisted protonation (X.sup(+) = H.sup(+); Fig. 6A). A subsequent dehydration would yield the oxazoline, now more committed to the heterocyclic fate. It is conceivable that X.sup(+) is the (gamma) -PO.inf(3).sup(-) from ATP, to yield a phosphate group as -OX, with subsequent elimination of P.inf(i) to form oxazoline. This would explain the ATP requirement and is a speculation ultimately testable with .sup(18)O-label in the Gly–Ser carbonyl oxygen. It is of note that the initial oxyanion cyclic adduct from Gly–Ser (or from Gly–Cys) is probably an intermediate common not only to the peptide-derived heterocyclic natural products but also in the pathways of protein autoproteolysis (B31) (B32) and protein self-splicing reactions (B33) . Residues Ser, Cys, or Thr are present at both splice junction sites of such proteins. Thus, as shown in Fig. 6B, net O-protonation routes the initial cyclic adduct toward five-ring heterocycle formation, whereas N-protonation sets the adduct up for C–N bond cleavage in net peptide bond fragmentation. The oxoester product can hydrolyze by means of acyl transfer to water in the autoproteolysis sequence, typical of sonic hedgehog (a Gly–Cys peptide bond is cleaved) (B32) or the proteosome precursors (a Gly–Thr peptide bond is cleaved) (B31) , or the product can undergo acyl transfer to an internal Ser or Cys side chain in the self-splicing sequences, for example, in RecA (B34) , Vent DNA polymerase (B35) , and TFP1 (B36) . Thus, the Gly–Ser (Gly–Cys) cyclization route may be rather ancient biochemistry with N-protonation as the default pathway, whereas routing of flux by O-protonation may require specific protein catalysis (for example, McbB or -D).

Several natural products, including thiostrepton, thiangazole, and patellamide (Fig. 1), stop at the dihydroaromatic five-membered thiazoline or oxazoline ring stage, presumably because of kinetic release before aromatization [which is also the case apparently for biogenesis of phleomycin, a thiazoline precursor to bleomycin (B37)] or because the biosynthetic enzymes lack a terminal dehydrogenase activity. A priori, we reasoned the loss of two hydrogens and two electrons would most likely be a proton/hydride desaturation and that a flavoprotein desaturase was a catalytic entity to anticipate on the basis of precedents for acyl coenzyme A desaturase (B38) , dihydroorate dehydrogenase (B39) , MurB (B40) , and proline dehydrogenase (B41) , all flavoenzymes. Indeed, our finding that McbC purifies with one equivalent of flavin mononucleotide (FMN) makes it a likely terminal desaturase, predictively funneling the electrons removed

from the (beta) carbon of the oxazoline as a hydride ion and then passing them on to O.inf(2) as cosubstrate for E (mid-dot) FMNH.inf(2) reoxidations, explaining the requirement for O.inf(2) and balancing the redox stoichiometry for each ring formed. We do not yet know for the eight ring-forming His.inf(6)-McbA or even in the two ring-forming McbA(1-46) the kinetic regioselectivity (that is, Ser before Cys as proposed in Fig. 6A) for ring formation or desaturation.

In sum, the ability to purify the McbB-, McbC-, and McbD-containing E. coli MccB17 synthase and the initial findings on mechanism, propeptide recognition, and cofactor requirements set the stage for structure and function studies to analyze the individual roles of these three proteins and their counterparts in other such heterocycle biosynthetic enzymes and to define the rules for recognition of peptide substrates.

Figure F1
Caption: Structures of bleomycin A.inf(2), thiostrepton, thiangazole, pristinamycin II.inf(A), and patellamide A. Thiazoline (dihydrothiazole) and thiazole rings are shown in red and oxazoline (dihydrooxazole) and oxazole rings are shown in blue.

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Figure F2
Caption: Structure of microcin B17 (MccB17) and its maturation pathway. MccB17 contains four thiazole (red) and four oxazole (blue) rings that are derived from the posttranslational modification of Cys (red) and Ser (blue) residues, respectively. The mcbA gene encodes a 69-amino acid precursor (pre-MccB17) that undergoes at least two steps of posttranslational modification. First, the products of the genes mcbB, -C, and -D mediate heterocycle formation to generate pro-MccB17. Subsequently, the NH.inf(2)-terminal 26 residues are removed, yielding mature MccB17. The Met residue at the NH.inf(2)-terminus of pre-MccB17was designated as the first residue in the numbering scheme used in this study. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys;D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Figure F3
Caption: (A) Representation of fusion proteins and peptides used as substrates or inhibitors of MccB17 synthase; C (Cys) and S (Ser) represent the residues that are posttranslationally modified to form the heterocycles; thus, McbA(1-46) has two modifiable residues and McbA(1-26) has none. McbA- (beta) -Gal was produced and purified as described (B42) . McbA(1-46), -(27-69), -(27-46), and -(1-26) were synthesized with a solid-phase synthetic strategy (B43) . The stippled region represents the propeptide sequence. (B) Time dependence of McbA- (beta) -Gal modification by MccB17. synthase. The reaction mixture contained 150 (mu) M McbA- (beta) -Gal, 50 mM tris-HCl (pH 7.5), 50 mM KCl, 2 mM MgCl.inf(2), 1 mM CaCl.inf(2), 1 mM adenosine triphosphate (ATP), 1 mM dithiothreitol (DTT), and cellular protein (3.5 mg/ml) [supernatant of a lysate from ZK4(pPY113) centrifuged at 15,000g]. The reaction was carried out at 37.Deg.C and stopped by adding SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer. Samples were analyzed by SDS-PAGE (7.5% polyacrylamide) followed by protein immunoblot analysis. The primary antibody was a polyclonal antibody specific for pro-and mature MccB17 (B14) , and the secondary antibody was goat antibody to rabbit immunoglobulin (**horseradish peroxidase** conjugate) (Pierce). The protein immunoblot was developed with the

SuperSignal reagent (Pierce).
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Figure F4
Caption: (A) Purified MccB17 synthase. MccB17 synthase was purified as described (B44) . (B) Co-immunoprecipitation of McbB, -C, and -D. The immunoprecipitates were probed with anti-McbB or anti-McbC. Lane 1, soluble fraction of ZK4(pPY113) before immunoprecipitation; lane 2, purified protein (McbB, upper panel and His.inf(6)-McbC, lower panel); lane 3, ZK4(pPY113) lysate precipitated with anti-McbB; lane 4, ZK4(pPY113) lysate precipitated with anti-McbC; and lane 5, ZK4(pPY113) lysate precipitated by anti-McbD. The three proteins McbB, His.inf(6)-McbC, and His.inf(6)-McbD, were individually overexpressed, purified to homogeneity (B24) , and used for antibody production (B45) . The soluble fraction (105,000g supernatant) of ZK4(pPY113) was separately incubated with anti-McbB (1:50 dilution), anti-McbC (1:100), and anti-McbD (1:25) at 4.Deg.C for 2 hours; then protein A-Sepharose (Pharmacia) was added and the mixture incubated for 1 hour (B45) .
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Figure F5
Caption: (A) MS analysis ([M + H].sup(+)) of the HPLC-purified products of MccB17 synthase action after 0 min and 45 min (B46) . The peaks (33% solvent B) from the 0-and 45-min reactions represent the starting material [McbA(1-46)] and the final product [Mcb(1-46) containing the 4,2-oxazole-thiazole moiety], respectively (B18) . (B) Summary of the MS analysis of substrates McbA(1-46) and His.inf(6)-McbA. The reactions with His.inf(6)-McbA as substrate were carried out as described above for Mcb(1-46) except that the time points analyzed were 0 and 60 min. His.inf(6)-McbA eluted from HPLC at 30% solvent B. As with Mcb(1-46), MS analysis of the HPLC peaks for the 0-and 60-min reactions indicate that the peaks represent the starting material and the final product (His.inf(6)-McbA containing four oxazoles and four thiazoles), respectively. Calc., calculated mass; Meas., measured mass.
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Figure F6
Caption: (A) Proposed mechanistic scheme for thiazole and oxazole formation in MccB17. Three steps are proposed: cyclization, dehydration, and dehydrogenation. (B) Common pathway of heterocycle formation and protein splicing and protein autoproteolysis.
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16. Plasmid pLarA-15b was generated by inserting the mcbA gene that was amplified by polymerase chain reaction (PCR) from pPY113 into the expression vector pET15b (Nde I-Bam HI sites) (Novagen). His.inf(6)-McbA expression was induced in BL21(DE3)(pLarA-15b) cells and purified by Ni.sup(2+)-chelate chromatography. The His.inf(6) tag adds 20 aa to the NH.inf(2)-terminus of McbA. Mass spectrometry and amino acid analysis indicated that the first Met was removed. ;
17. Aminoethyl bithiazole carboxylic acid was cross-linked to BSA by disuccinimidyl suberate through primary amines. Protein immunoblot analysis showed that the cross-linked sample was recognized by anti-MccB17, whereas control samples (BSA alone and BSA plus disuccinimidyl suberate) were not. ;
18. Y.-M. Li, J. C. Milne, L. L. Madison, R. Kolter, C. T. Walsh, data not shown. ;
19. The plasmids pChenB, pChenC, and pChenD contain gene-disrupting insertions [mini Tn10 kanamycin resistance (kan.sup(r))] in mcbB, mcbC, and mcbD of pCID909 (B12) , respectively (T. Chen and R. Kolter, unpublished results). The parent pCID909 and knockout plasmids pChenB, pChenC, and pChenD were transformed into ZK4 cells [L. Gilson, H. K. Mahanty, R. Kolter, J. Bacteriol. 169, 2466 (1987)], and the cellular extracts were used for an in vitro assay as described in Fig. 3B. ;
20. ZK4(pPY113) containing the seven genes mcbA through mcbG cloned into pBR322 (B10) was used for MccB17 synthase production. ;
21. Protein amounts were determined with the Bradford reagent (Bio-Rad). McbA- (beta) -Gal was used as substrate of MccB17 synthase. A time course of the modification was monitored by protein immunoblot and quantitated by the NIH-Image program. A point in the linear range was used to calculate the specific activity. The uncalibrated optical density was used for the area determination in the same blot. ;
22. The k.inf(cat) for McbA(1-46) was estimated from the linear portion of rate plots as in Fig. 3B. Conversion of the absorbance units in the immunoblot to picomoles of bisheterocycle product was obtained from endpoint absorbance where MS analysis revealed only bisheterocycle product. ;
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knockout strain of E. coli (MC4100 (Delta) htpG) indicated no effect on MccB17 production assessed by a bioassay (B9) and at most a 50% decrease in the rate of posttranslational modification of McbA- (beta) -Gal in vitro (B18) . Therefore, the role of HtpG in MccB17 maturation remains uncertain. MC4100 (Delta) htpG was constructed by P.inf(1) transduction of zba-315::kan, (Delta) htpG::lacZ from JCB42 [J. C. A. Bardwell and E. A. Craig, J. Bacteriol.170, 2977 (1988)]. The htpG knockout strain MC4100 (Delta) htpG and control strain MC4100 were transformed with plasmid pPY113. These strains were assayed as described in Fig. 3B except that the McbA- (beta) -Gal concentration was 30 (mu) M. ;

24. Plasmid pLarB-11d was generated by insertion of the mcbB gene into the expression vector pET-11d-CKII (beta) a (Nde I-Bam HI sites) [Y. Shi, E. D. Brown, C. T. Walsh, Proc. Natl. Acad. Sci. U.S.A. 91, 2767 (1994)]. Plasmids pLarC-15b and pLarD-15b were constructed by cloning mcbC and mcbD into the expression vector pET-15b (Nde I-Bam HI sites), respectively. All three genes were amplified by PCR from pPY113 with the specific primers with restriction sites (Nde I or Bam HI). McbB was purified from DE3(BL21)(pLarB-11d) by five-step purification: Q-Sepharose, ammonium sulfate precipitation, gel filtration, Phenyl-Sepharose, and MonoQ. His.inf(6)-McbC was purified from a strain of DE3(BL21)pLysS(pLarC-15b) by Ni.sup(2+)-chelate chromatography. His.inf(6)-McbD was purified from inclusion bodies of DE3(BL21) pLysS(pLarD-15b) by affinity chromatography and SDS-PAGE. ;

25. Attempts to effect modification of McbA(27-69) or Mcb(27-46) by adding McbA(1-26) in trans have not resulted in heterocycle formation. Combinations of three concentrations (9.6, 2.4, and 0.6 (mu) M) of McbA(1-26) and four concentrations (135, 24, 85, and 2.1 (mu) M) of McbA(27-69) were used to test the two peptides in trans for heterocycle formation. The assay conditions were as described in Fig. 5A. The reactions at 0 and 120 min were stopped and analyzed by protein immunoblot. ;

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28. In vivo data to be presented elsewhere (B15) on the McbA- (beta) -Gal fusion construct further confirms that the (1-26) propeptide region is a major recognition determinant for MccB17 synthase. ;

29. Reaction components were degassed separately in reactivials (Pierce) fitted with silicon-teflon septa on a vacuum line by repeated evacuation and backfilling with argon (for anaerobic reactions) or air (for control reactions) (10 times). The vials were then incubated on ice for 30 min, and the degassing procedure was repeated two more times. Anaerobic modification reactions were carried out in a controlled atmosphere glove box (Model 50004; Labconco) that had been evacuated and backfilled with argon five times, and control reactions were carried out on the bench top. The modification reactions were carried out essentially as described above for Mcb(1-46). To assess the level of oxygen remaining after the degassing procedure, the activity of glucose oxidase was determined. Glucose oxidase catalyzes the oxidation of (beta) -d-glucose using O.inf(2) as the hydrogen acceptor. Glucose oxidase activity was determined with the glucose oxidase-peroxidase-o-dianisidine assay system [H. U. Bergmeyer, Methods of Enzymatic Analysis (Academic Press, New York, 1974), vol. 1, p. 457]. The reaction mix contained 0.04 units of glucose oxidase (Calbiochem), 0.4 units of **horseradish peroxidase** (Calbiochem), 0.37 M (beta) -d-glucose, o-dianisidine (0.4 mg/ml) (Sigma), and 0.1 M sodium phosphate (pH 7.0). The reaction was initiated

by adding glucose oxidase to the reacation mix. Samples were removed at 4, 8, 12, and 16 min, the reaction was quenched with HCl, and the absorbance at 460 nm was then determined. ;
30. The optical spectrum of purified McbC indicated the presence of a flavin moiety. The flavin was released from the enzyme by boiling for 15 min. The denatured protein was removed by centrifugation, and the flavin was identified by HPLC analysis (Vydac C18 protein-peptide column) with a gradient of methanol (10 to 40% in 60 min) in 0.1 M potassium phosphate (pH 5.3). ;
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42. The Sal I-Xmn I fragment of mcbA was used to generate an in-frame fusion with (beta) -Gal at the 65th codon of mcbA, creating the plasmid pPY116 (B15) . McbA- (beta) -Gal was purified from the strain ZK4(pPY116) with an aminobenzyl 1-thio- (beta) -d-galactopyranoside-agarose column (Sigma). ;
43. All four peptides were purified by HPLC [Bio-Rad Preparative C18 column, with solvent A: 0.1% trifluoroacetic acid (TFA) in water, and solvent B: 0.1% TFA in acetonitrile] and the structure confirmed by mass spectrometry. The concentration of the peptides was determined by amino acid analysis. ;
44. The ZK4(pPY113) strain was used for purification of MccB17 synthase. An overnight culture grown in LB media containing ampicillin (Amp) was diluted 500-fold in M63 medium containing Amp and grown at 37.Deg.C for 20 to 24 hours. The cells were pelleted by centrifugation (5000g) and lysed with a French pressure cell. The supernatant after 105,000g centrifugation was used for purification by phenyl-Sepharose, DEAE-Sepharose, Sephacryl S-200, and MonoQ (Pharmacia). The activity was monitored by protein immunoblot analysis as described in Fig. 3, with McbA- (beta) -Gal as a substrate. ;
45. Polyclonal rabbit antibodies to each protein were generated by East-Acres Biologicals. Protein immunoblot analysis shows that the antisera specifically recognize their antigen and do not cross-react with other proteins (B18) . The immunocomplexes were washed with buffer [50 mM tris-HCl (pH 7.5), 100 mM KCl, 4 mM MgCl.inf(2), 2 mM CaCl.inf(2)] four times and collected by centrifugation. SDS sample buffer was added to the

complex, and the supernatants were subjected to protein immunoblot analysis. ;
46. Reaction mixtures consisted of 7 (mu) M McbA(1-46), 50 mM tris-HCl (pH 7.5), 50 mM KCl, 2 mM MgCl.inf(2), 1 mM CaCl.inf(2), 1 mM ATP, 1 mM dithiothreitol, and purified MccB17 synthase (0.24 mg/ml). The reactions were carried out at 37.Deg.C and stopped by injection onto HPLC (Vydac C18 column, 300 angstrom , solvent A: 0.1% TFA in water, and solvent B: 0.09% TFA in acetonitrile). McbA(1-46) was eluted around 33% solvent B. The 0-and 45-min reaction mixtures showed the same elution patterns, a peak at 33% solvent B and other protein peaks after >45% solvent B. HPLC fractions were analyzed by protein immunoblot with anti-MccB17 and only the peak (33% solvent B) for the 45-min reaction was antibody-positive (B18) . ;
47. We thank C. E. Dahl of the Department of Biological Chemistry and Molecular Pharmacology of the Biopolymers Facility for synthesis of the microcin peptides; I. Jenson and M. Obenauer of Howard Hughes Medical Institute Research Laboratory for NH.inf(2)-terminal sequencing, amino acid analysis, and MS analysis; S. Hecht (University of Virginia) for aminoethyl bithiazole carboxylic acid; M. Bollinger (Pennsylvania State University) and S. Fisher and D. McCafferty of this laboratory for their help and suggestions regarding the anaerobic reactions. We also thank other members of the C.T.W. laboratory for suggestions concerning this manuscript. Supported by NIH grant GM20011 (C.T.W.) and NSF grant MCB9207323 (R.K.). J.C.M. is supported by a postdoctoral fellowship (grant PF4332) from the American Cancer Society, and L.L.M. is supported by NIH training grants 5T32GM07306-19 and 5T32AI07410-03.
Descriptors: Biochemistry

(THIS IS THE FULLTEXT)

...Text: pro-and mature MccB17 (B14) , and the secondary antibody was goat antibody to rabbit immunoglobulin (**horseradish peroxidase** conjugate) (Pierce). The protein immunoblot was developed with the SuperSignal reagent (Pierce...

References and Notes:
...U.S.A. 84, 5177 (1987). We hypothesized that HtpG could possibly function as a **chaperone** in McbA recognition, but subsequent analysis of an htpG knockout strain of E. coli (MC4100...The reaction mix contained 0.04 units of glucose oxidase (Calbiochem), 0.4 units of **horseradish peroxidase** (Calbiochem), 0.37 M (beta) -d-glucose, o-dianisidine (0.4 mg/ml) (Sigma), and...

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DIALOG(R)File 370:Science

00500729 (THIS IS THE FULLTEXT)
Requirement for Invariant Chain in B Cell Maturation and Function
Shachar, Idit; Flavell, Richard A.
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Abstract: Previously the role of invariant chain (Ii) had been described only as a **chaperone** that facilitates folding and transport of major histocompatability complex class II molecules; here it is shown that Ii is required for B cell development. B cells from mice lacking Ii were found to have a low response to T-independent type II antigen and could not proliferate after the mice were injected with antigen. Study of cell surface markers revealed a developmental arrest that prevented immature virgin B cells from becoming mature B cells in the periphery. This block was independent of major histocompatability complex class II expression and was an intrinsic feature of B cells that correlated with the amount of Ii. Thus, Ii participates by an unknown mechanism in B cell maturation.

Text: Major histocompatability complex (MHC) class II molecules associate with trimers of Ii during biosynthesis. Ii facilitates folding of class II molecules, interferes with their association with peptides, and is involved in MHC class II transport (B1) . Furthermore, elimination of the Ii gene by gene targeting greatly diminishes the ability of antigen-presenting cells (APCs) to present exogenous protein antigen in a class II-restricted fashion and impairs the maturation of CD4.sup(+) T cells in the thymus (B2) (B3) (B4) . The assembly, transport, and function of MHC class II have been studied in detail in mice lacking Ii (B2) (B3) (B4) . There has not, however, been a rigorous examination of the functional capability of B cells. We therefore analyzed the function of B cells lacking Ii.

To examine the function of B cells lacking Ii (Ii.sup(-)), we measured B cell response both to the type II thymic-independent (TI) antigen NP-Ficoll and to NP-CGG, a thymic-dependent (TD) antigen (Fig. 1). Both TD and TI responses were markedly reduced in the Ii.sup(-) mice. The Ii.sup(-) mice have reduced numbers of CD4.sup(+) T cells, which predicts that these mice should have weak responses to TD antigen. However, unlike the response in class II-deficient mice (B5) (B6) , 6 days after immunization, concentrations of immunoglobulin M (IgM) to NP were low, suggesting that the primary response of B cells was also impaired. This observation is consistent with the defective primary antibody response by the Ii.sup(-) mice after keyhole limpet hemocyanin (KLH) injection (B2) . In response to NP-Ficoll, B cells lacking Ii produced little IgM both 6 and 14 days after injection. Thus, the B cells in Ii.sup(-) mice were unable to respond normally to TI antigen (Fig. 1).

Equivalent numbers of B220.sup(+) B cells were found in the periphery of the control mice, Ii.sup(-) mice, and two lines of transgenic Ii mice that express low amounts of one of the two isoforms of Ii, p31 and p41 (designated Ii.sup(p31lo) and Ii.sup(p41lo), respectively) (B7) (B8) . To determine the ability of these B cells to respond to antigen in vivo after stimulation, we immunized mice with KLH and examined draining lymph node B cells 9 days later. In the draining lymph nodes of control mice, the B220.sup(+) B cell population had proliferated and increased to 54.3 +/- 6.8% of the total cells from 13.75%. In the absence of Ii or in the presence of low amounts of p31 or p41 Ii, however, the B cell population expanded to only 26.6 +/- 3.2% (B9) (B10) . Because in both Ii.sup(p31lo) and Ii.sup(p41lo) mice CD4.sup(+) T cells are present in normal amounts (B7) , this low proliferation cannot be attributed to the CD4.sup(+) T cell

deficiency. Thus, the low number of B cells found after immunization with protein antigen could be explained by a defect in the B cell response or by the rapid death of these cells. B cells from Ii.sup(-) mice proliferated as well in vitro as did wild-type cells in the presence of lipopolysaccharide (LPS) (B11) .

B cell development occurs independently of MHC class II expression (B5) (B6) . To analyze B cell maturation in the absence of Ii, we compared spleen cells from control and Ii knockout mice (Ii.sup(-)) using a panel of antibodies to B cell markers. Unlike B cells from control mice, the Ii.sup(-) B cells expressed lower amounts of CD23 and higher amounts of IgM and heat-stable antigen (HSA) (Fig. 2A). Thus, peripheral B cells in the absence of Ii were immature. Analysis of B cells in the periphery of the Ii.sup(p31lo) and Ii.sup(p41lo) transgenic mice revealed that the low amounts of Ii that could restore T cell-positive selection (B7) (B12) could not mediate full B cell maturation in the periphery (B13) . Cell surface markers expressed on these B cells remained unchanged 9 days after KLH injection (Fig. 2B). Thus, B cells in the absence or presence of low amounts of Ii remained of an immature phenotype. B cells that could proliferate in vitro after LPS stimulation remained immature as well (B14) .

To investigate whether the absence of conventional MHC class II was responsible for this B cell maturation defect, we examined maturation markers on B cells from class II-deficient mice. B cells from mice lacking MHC class II (MHC.sup(-)) (B5) (B6) and from mice lacking the MHC class II transactivator (CIITA.sup(-)) (B15) had only slightly lower numbers of CD23.sup(hi), IgM.sup(int), and IgD.sup(hi) than did cells from wild-type mice (Fig. 2C). Therefore, the low numbers of CD23.sup(hi), IgD.sup(hi), and IgM.sup(int) cells in the periphery of the Ii.sup(-) B cells were not due to less conventional MHC class II expression or CD4.sup(+) T cells, but to the lack of Ii itself or perhaps a secondary consequence of a lack of Ii. Spleen cell lysates from the different mice were analyzed for Ii chain, and the p31 and p41 transgenic mice expressed low amounts of Ii (B7) . However, because CIITA deficiency only partially reduced Ii mRNA levels, the CIITA.sup(-) cells expressed about 80% of the Ii of cells from control mice (Fig. 2D). Therefore, the invariant chain, rather than MHC class II, determined the degree of maturation of virgin B cells to mature cells.

To determine the role of Ii in early B cell development, we examined bone marrow from Ii.sup(-) and control mice cytofluorometrically for the expression of surface antigens that correlate with specific stages in B cell ontogeny. The percentage of B220.sup(+) cells appeared to be slightly lower and the relative proportion of CD43.sup(+)B220.sup(+) cells slightly higher in bone marrow of Ii.sup(-) mice. As expected from their absence in the periphery, B220.sup(+)CD23.sup(+) cells were absent from Ii.sup(-) bone marrow. This CD23.sup(+) population might be cells that recirculate from the periphery to this compartment. This places the developmental block between the immature CD23.sup(lo), IgM.sup(hi), IgD.sup(lo), HSA.sup(hi) stage and the mature CD23.sup(hi), IgM.sup(int), IgD.sup(hi), HSA.sup(int) stages (Fig. 3A).

To determine if the lack of maturation was due to an intrinsic B cell defect or if the bone marrow epithelial or peripheral stromal cells failed to provide the signals required for B cell maturation, we isolated day 17 fetal liver cells from either control or Ii.sup(-) embryos and transferred them into irradiated control or Ii.sup(-) recipients. In control chimeric mice in which the recipients and the donors were Ii.sup(+), peripheral CD4.sup(+) and CD8.sup(+) T cells were reconstituted at normal levels and a mature normal B cell population was detected as expected (Fig. 3B, panels a, e, and i). In contrast, the irradiated Ii.sup(-) recipient mice that

received control (Ii.sup(+)) fetal liver cells reconstituted low amounts of CD4.sup(+) T cells (Fig. 3B, panel d). However, their B cells recovered a mature phenotype (Fig. 3B, panels h and l). Normal mice that were reconstituted with Ii.sup(-) cells showed a normal CD4.sup(+) T cell population (B16) (B17) (Fig. 3B, panel b), but their B cell population remained immature (Fig. 3B, panels f and j), showing the same phenotype as B cells of Ii.sup(-) mice (Fig. 3B, panels g and k) (B10) . Thus, unlike T cells, the inability of Ii.sup(-) B cells to mature is an intrinsic feature. Furthermore, B cells from CD4-deficient or interleukin-4 (IL-4)-deficient mice matured normally. Therefore, B cell maturation could progress in the absence of CD4.sup(+) T cells and IL-4, suggesting that these features do not explain the B cell defect seen in Ii.sup(-) mice (B18) .

Thus, we have shown that Ii is critical for B cell maturation. Until now Ii was characterized as a **chaperone** that participates in antigen processing by allowing MHC class II folding, maturation, and transport. We now show that the development from immature to mature B cells is a controlled process that needs a signal to occur. This step is controlled by Ii, and in the absence of this chain, B cells cannot mature or participate efficiently in the immune response.

Figure F1
Caption: Immunoglobulin M response to TD and TI antigens. Ii.sup(-) (circles) or wild-type littermates (triangles) were challenged withthe type II-TI antigen NP.inf(90)-Ficoll or with the TD antigen NP.inf(16)-CGG. At the indicated times after injection, blood was drawn and IgM titers were quantitated by enzyme-linked immunosorbent assay (B21) . The concentration of antibodies at time zero in the absence of immunization probably represents basal concentrations of low-affinity IgM, although it is not clear why this amount is higher in the Ii.sup(-) mice than in the control mice. Mean titers (micrograms per milliliter) were as follows. Day 0: control, 1.415 +/- 0.96 (n = 6); Ii.sup(-), 10.7 +/- 7.9 (n = 7). Day 6: control, 50.6 +/- 14.6 (n = 6); Ii.sup(-), 17.2 +/- 7.06 (n = 10). Day 14: control, 43.8 +/- 7.5 (n = 6); Ii.sup(-), 19.85 +/- 7.75 (n = 7). The symbol that appears solid represents two Ii.sup(-) mice with identical antibody concentrations.

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Figure F2
Caption: Cytofluorometric analysis of different maturation markers on B220 cells. (A) Control and Ii.sup(-) splenocytes were double-stained with antibody to B220 (anti-B220) and anti-IgM, anti-HSA, or anti-CD23. Histograms show expression of these molecules on B220 cells (B19) . (B) Nine days after KLH injection, lymph node cells from 6-to 8-week-old control and Ii.sup(-) mice were harvested and were stained by FACS for anti-B220 and anti-IgM, anti-HSA, or anti-CD23. FACS analysis of the different markers on B220.sup(+) cells is shown (B19) (B20) . (C) Control, Ii.sup(-), MHC.sup(-), or CIITA.sup(-) mice were triple-stained with anti-B220, anti-IgM, and either anti-CD23 or anti-IgD. The FACS analysis shows the different markers on B220.sup(+) cells (B19) . (D) Steady state concentrations of Ii. The Ii p31 and p41 isoforms are indicated (B22) . Con, control.

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Figure F3

Caption: (A) Cytofluorometric analysis of different maturation markers on B220.sup(+) cells in bone marrow. Bone marrow cells from control and Ii.sup(-) animals were triple-stained with anti-B220, anti-IgM, anti-CD23, anti-CD43, or anti-I-A.sup(b). The FACS analysis shows the different markers on B220.sup(+) cells (B19) . (B) T cell (top row) and B cell (middle and bottom rows) profiles from chimeric mice after fetal liver cell transfer. B6 (B6c) (panels a, e, i, d, h, and l) or Ii.sup(-) (Iic.sup(-)) (panels b, f, j, c, g, and k) fetal liver cells were transferred to B6 (B6m) (panels a, b, e, f, i, and j) or Ii.sup(-) (Iim.sup(-)) (panels c, d, g, h, k, and l) irradiated mice (B23) . Lymph node T cells (panels a to d) and B cells (panels e to l) were prepared and stained with antibodies to B220, CD4, CD8, IgM, IgD, and CD23.

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8. The Ii.sup(p31lo) and Ii.sup(p41lo) mice were obtained by crossing transgenic mice expressing low amounts of one Ii isoform with Ii.sup(-) mice to generate Ii.sup(-) mice expressing exclusively p31 or p41. B220.sup(+) B cells were examined in spleen and lymph nodes in the different mice. Lymph node and spleen cells from wild-type, Ii.sup(-), Ii.sup(p31lo), and Ii.sup(p41lo) mice were stained with antibody specific for B220.sup(+) and analyzed by fluorescence-activated cell sorting (FACS) (B19) . Percentage of B cells in spleen were as follows: control, 46.5 +/- 8.2; Ii.sup(-), 47 +/- 10.9; Ii.sup(p31lo), 43.22 +/- 9.1; and Ii.sup(p41lo), 43.42 +/- 6.07. Percentage of lymph node cells were as follows: control 13.75 +/- 5.6; Ii.sup(-), 15.07 +/- 5.5; Ii.sup(p31lo), 12 +/- 5.3; and Ii.sup(p41lo), 9 +/- 1. ;
9. Lymph node cells from 6-to 8-week-old control, Ii.sup(-), Ii.sup(p31lo), or Ii.sup(p41lo) mice were harvested and were stained by FACS for B220.sup(+) expression 9 days after KLH injection (B19) (B20) . ;
10. After the fetal liver adoptive-transfer experiment (Fig. 3B), we immunized mice with KLH (B20) and found lower amounts of B220.sup(+) B cells when Ii.sup(-) cells were transferred, suggesting that the lower amounts of B220.sup(+) cells are due to an intrinsic property of these B cells. ;
11. Purified B cells from control or Ii.sup(-) mice were cultured at a density of 2 x 10.sup(5) cells per 100 (mu) l in Bruff's medium supplemented with 5% fetal bovine serum (FBS), penicillin-streptomycin (100 U/ml), and several concentrations of LPS. DNA synthesis was assayed by pulsing the cultures with 1.0 (mu) Ci [.sup(3)H]thymidine per well at 48 hours and then incubating them for an additional 12 hours, after which the cells were harvested and counted on a scintillation counter. Assays were done in triplicate. Proliferation was determined at 48 hours by [.sup(3)H]thymidine incorporation for 12 hours. ;
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13. For cytofluorometric analysis of different maturation markers on B220.sup(+) cells, splenocytes from Ii.sup(p31lo) and Ii.sup(p41lo) mice were double-stained with anti-B220 and anti-IgM, anti-HSA, or anti-CD23 (B19) . The markers expressed on the B cells from these mice were almost identical to those expressed on splenocytes from Ii.sup(-) mice. ;
14. Purified B lymphocytes from wild-type or Ii.sup(-) mice were cultured in the presence of different concentrations of LPS. Cells were then double-stained with anti-B220 and anti-IgM, anti-HSA, or anti-CD23. B cells from Ii.sup(-) mice expressed immature markers after LPS stimulation. ;
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18. Control, Ii.sup(-), CD4.sup(-), or IL-4.sup(-) mice were triple-stained with anti-B220, anti-IgM, or anti-CD23 (B19) . The FACS analysis showed that B cells from the CD4.sup(-) or IL-4.sup(-) mice expressed almost identical amounts of the markers as did B cells from wild-type mice. ;
19. The following antibodies used in experiments were purchased from Pharmingen: AF6-120.1 for I-A.sup(b); H129.1.9 for CD4, 53-6.7 for CD8, M1/69 for heat-stable antigen (HSA), S7 for CD43, B3B4 for CD23, 14.8 for CD45RA (B220), R6-60.2 for IgM, and AMS 9.1 for IgD. Bone marrow, lymph nodes, and spleen cell suspensions were prepared in Bruff's medium, and the spleen erythrocytes were lysed by hypotonic shock. Cells were resuspended in cold phosphate-buffered saline (PBS) supplemented with 1% FBS. Staining was performed in the same buffer. ;
20. Mice were injected in the hind footpads with 100 (mu) g of KLH emulsified in complete Freund's adjuvant. Draining lymph nodes were collected 9 days later. ;
21. Control and experimental animals 6 to 8 weeks of age were injected intraperitoneally with 25 (mu) g of NP.inf(90)-Ficoll (NP-AECM-ficoll, Biosearch) in 0.1 ml of 0.85% NaCl. Immunization with NP.inf(16)-CGG was with 100 (mu) g of Alum-precipitated NP-CGG in 0.1 ml of 0.85% NaCl. ;
22. Splenocytes from wild-type, Ii.sup(-), or CIITA.sup(-) mice were incubated in digitonin (50 (mu) g/ml). The pellet was then lysed in 0.5% Triton X-100, 300 mM NaCl, 50 mM tris (pH 7.4), 1 mM polymethylsulfonyl fluoride, leupeptin (10 (mu) g/ml), aprotinin (10 (mu) g/ml), pepstatin (10 (mu) g/ml), chymostatin (10 (mu) g/ml), and 20 mM N-ethyl-maleimide. Nuclei and debris were eliminated by centrifugation. Lysates were separated on 12% (w/v) SDS-polyacrylamide gel electrophoresis. The proteins were transferred onto nitrocellulose, which was blocked and then incubated with IN1 (monoclonal antibody to the Ii cytoplasmic tail) followed by **horseradish peroxidase** -conjugated goat antibody to rat IgG. ;
23. Recipient animals were irradiated with 9.2 Gy delivered by a cesium source. Fetal liver cells from mice after 16 days of gestation were prepared and 5 x 10.sup(6) cells were injected intravenously. ;
24. Supported by the Howard Hughes Medical Institute (R.A.F.) and the Irvington Institute (I.S.). We thank T. Geiger, I. S. Grewal, C.-H. Chang, P. Cresswell, M. Shlomchik, and L. Cohn.
Descriptors: Immunology

(THIS IS THE FULLTEXT)

Abstract: Previously the role of invariant chain (Ii) had been described

only as a **chaperone** that facilitates folding and transport of major histocompatability complex class II molecules; here it is...

...Text: that Ii is critical for B cell maturation. Until now Ii was characterized as a **chaperone** that participates in antigen processing by allowing MHC class II folding, maturation, and transport. We...

References and Notes:
...blocked and then incubated with IN1 (monoclonal antibody to the Ii cytoplasmic tail) followed by **horseradish peroxidase** -conjugated goat antibody to rat IgG...

6/9,K/70 (Item 5 from file: 370)

00500561 (THIS IS THE FULLTEXT)
Support for the Prion Hypothesis for Inheritance of a Phenotypic Trait in Yeast
Patino, Maria M.; Liu, Jia-Jia; Glover, John R.; Lindquist, Susan
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Abstract: A cytoplasmically inherited genetic element in yeast, [PSI.sup(+)], was confirmed to be a prionlike aggregate of the cellular protein Sup35 by differential centrifugation analysis and microscopic localization of a Sup35-green fluorescent protein fusion. Aggregation depended on the intracellular concentration and functional state of the **chaperone** protein Hsp104 in the same manner as did [PSI.sup(+)] inheritance. The amino-terminal and carboxy-terminal domains of Sup35 contributed to the unusual behavior of [PSI.sup(+)]. [PSI.sup(+)] altered the conformational state of newly synthesized prion proteins, inducing them to aggregate as well, thus fulfilling a major tenet of the prion hypothesis.

Text: Mammalian prions cause devastating neurodegenerative disorders (B1) . Unlike conventional pathogens, they are thought to consist entirely of protein-specifically, a normal nuclear-encoded protein, PrP.sup(C), with an altered "scrapie" conformation, PrP.sup(Sc) (B1) . The key to prion pathology is thought to be the ability of PrP.sup(Sc) to induce new PrP.sup(C) molecules to adopt the altered structure, producing a protein-conformation cascade that causes the disease and gives rise to new infectious PrP.sup(Sc).

A similar explanation can account for the otherwise baffling behavior of two genetic factors in yeast, [PSI.sup(+)] and [URE3] (B2) . The [PSI.sup(+)] factor increases translational read-through of all three nonsense codons, and is monitored in the laboratory by omnipotent suppression of nonsense mutations (B3) . Although unlinked to any known

nucleic acid, [PSI.sup(+)] behaves as a dominant, cytoplasmically inherited genetic element. It bears an unusual relation to the nuclear-encoded protein Sup35 that is reminiscent of the relation between mammalian prions and nuclear-encoded PrP.sup(C) (B1) (B2) (B3) (B4) .

Normally, Sup35 is a subunit of the translation-release factor that causes ribosomes to terminate translation at nonsense codons. Release activity maps to the COOH-terminal domain (B5) , which is essential for growth (B6) . Sup35's NH.inf(2)-terminal domain is not essential and is required only for the propagation of [PSI.sup(+)] (B6) . Mutations in Sup35 can also cause omnipotent nonsense suppression, but unlike [PSI.sup(+)], the mutant phenotypes exhibit Mendelian inheritance (B3) . Remarkably, transient overexpression of Sup35, or just its NH.inf(2)-terminal domain, can induce de novo heritable [PSI.sup(+)] elements (B2) (B6) . Moreover, transient overexpression of the **chaperone** Hsp104 can restore translational fidelity, heritably converting cells from [PSI.sup(+)] to [psi.sup(-)] (B4) .

These observations argue that [PSI.sup(+)] represents the inheritance of a self-perpetuating alteration in the conformation of Sup35, which is initiated by the NH.inf(2)-terminal domain and impairs the ability of the COOH-terminal domain to function in translation. Although this mechanism successfully explains many perplexing genetic observations (B2) (B3) , such a revolutionary model for the inheritance of a phenotypic trait demands the support of direct physical evidence, which we provide here.

Insolubility of Sup35 in [PSI.sup(+)] cells. Isogenic [psi.sup(-)] and [PSI.sup(+)] strains of two different genetic backgrounds (B7) contained the same quantity of Sup35 and Sup45 (Fig. 1 A), the other subunit of the translation-release factor (B8) . Thus, the read-through of nonsense codons in [PSI.sup(+)] cells was not due to reduced accumulation of the termination-factor subunits. Nor was it likely due to posttranslational modification. The migration of the Sup35 and Sup45 proteins from [PSI.sup(+)] cells on high resolution two-dimensional gels was identical to that of the proteins from [psi.sup(-)] cells (B9) .

In contrast, the solubility of Sup35 was very different in [PSI.sup(+)] and [psi.sup(-)] cells. Most Sup35 protein in [PSI.sup(+)] lysates pelleted after centrifugation at 12,000g; most remained in the supernatant of [psi.sup(-)] lysates. In [psi.sup(-)] lysates, a substantial fraction of Sup35 remained soluble after centrifugation at 100,000g; none remained soluble in [PSI.sup(+)] lysates (Fig. 1B). Similar differences in the solubility of Sup35 in [PSI.sup(+)] and [psi.sup(-)] cells were obtained in the early, mid-, and late log phases of growth as well as in cells in the stationary phase (Fig. 1C) (B10) . No difference in the sedimentation properties of total proteins was detected by Coomassie blue staining, nor did immunostaining show any difference in the sedimentation of Sup45, ribosomal protein L3, or the **chaperone** proteins Hsp70, Hsp90, and Hsp104 (Fig. 1, B and C) (B10) . High salt (1 M KCl), EDTA (50 mM), and ribonuclease A (400 (mu) g/ml) treatments did not reduce the quantity of Sup35 found in the pellet of [PSI.sup(+)] cells, nor did treatments with nonionic detergent (1% Triton X-100) (B10) . Moreover, like PrP.sup(Sc) (B1) , the Sup35 protein found in these aggregates was resistant to proteolysis (B11) .

Role of the **chaperone** Hsp104 in Sup35 aggregation. Overexpression of Hsp104, a protein that promotes the resolubilization and reactivation of heat-damaged proteins (B12) , converts cells from [PSI.sup(+)] to [psi.sup(-)] (B6) . If aggregates of Sup35 reflect the presence of [PSI.sup(+)], Sup35 should return to the soluble state after this conversion. When cells were transformed with a centromeric vector expressing Hsp104 from its own promoter, this was indeed the case (Fig.

1D). [In this and all experiments reported here, the [PSI.sup(+)] and [psi.sup(-)] states were confirmed by plating assays on selective media (Fig. 2) (B13) .] A stronger test of the relation between Sup35 aggregates and [PSI.sup(+)] derives from the ability of transient Hsp104 overexpression to heritably cure cells of [PSI.sup(+)] (B6) . A galactose-inducible single-copy vector, which provides uniform expression in all cells, converted more than 80% of [PSI.sup(+)] cells to [psi.sup(-)] after 3.5 hours of galactose induction. In converted cells plated to glucose media without continued selection for the Hsp104 plasmid (B13) , Sup35 was found in the soluble fraction, even though Hsp104 was no longer overexpressed (B10) . Similar results were obtained with a glucocorticoid-inducible Hsp104 expression vector (B10) .

Paradoxically, [PSI.sup(+)] cells also convert to [psi.sup(-)] when Hsp104 expression is eliminated by deletion of the HSP104 gene (B6) . This is surprising because the only previously known function of Hsp104 was to promote the dissolution of aggregates of heat-damaged proteins; these aggregates are maintained in cells with HSP104 deletions (B12) . When [PSI.sup(+)] cells were converted to [psi.sup(-)] through deletion of HSP104, Sup35 was found in the soluble fraction (Fig. 1E). Thus, the behavior of Sup35 aggregates paralleled the behavior of [PSI.sup(+)] and differed from the behavior of heat-damaged aggregates. Together these data strongly support the hypothesis that [PSI.sup(+)]-mediated nonsense suppression is due to a conformational alteration in Sup35 that is self-sustaining as long as Hsp104 is present at its normal concentration.

The sedimentation properties of Sup35 also provide a simple molecular explanation for one of the most perplexing aspects of [PSI.sup(+)] biology-the ability of the element to exist in a cryptic form. For example, when [PSI.sup(+)] cells were transformed with plasmids encoding mutations in the Hsp104 nucleotide-binding domains (NBDs) [either K218T (a Lys to Thr substitution at amino acid 218 in NBD 1) or K620T (an analogous substitution in NBD 2)], the [PSI.sup(+)] phenotype was suppressed but not cured (B4) (Fig. 2B). That is, the cells did not exhibit nonsense suppression and were unable to grow on selective media, but when the plasmid encoding the mutant Hsp104 protein was lost, [PSI.sup(+)] reappeared and growth on selective media was restored. In contrast, the double mutant K218TK620T cured cells of [PSI.sup(+)]; when the expression plasmid was lost, [PSI.sup(+)]-mediated nonsense suppression was not regained (Fig. 2B).

As shown in Fig. 1F, a greater fraction of Sup35 remained soluble in cells expressing K218T or K620T than in the original [PSI.sup(+)] strain, but most of the the protein remained insoluble (Fig. 1F). Presumably, the increase in soluble Sup35 allowed faithful termination at nonsense codons, but a sufficient quantity of aggregated Sup35 remained to reestablish [PSI.sup(+)] when the plasmid encoding the mutant protein was lost. A smaller fraction of Sup35 was insoluble in the double mutant, and this material was unable to act as a prion-inducing element.

Visualizing prion elements. To monitor [PSI.sup(+)] elements in real time in living cells, we used a green fluorescent protein (GFP) fusion (B14) . The NH.inf(2)-terminal prion-determining domain (NPD) of Sup35 was fused to GFP and placed under the control of the regulatable promoters CUP1 (inducible with copper) and GRE [inducible with 11-deoxycorticosterone (DOC)] (B15) . When NPD-GFP was induced by either copper or DOC, fluorescence was diffusely distributed in two different [psi.sup(-)] strains (B7) . In their isogenic [PSI.sup(+)] derivatives, as soon as fluorescence could be detected it was concentrated in a small number of intense foci (Fig. 3 A). When subjected to differential centrifugation, NPD-GFP sedimented with Sup35 in [PSI.sup(+)] lysates but remained in the

supernatant of [psi.sup(-)] lysates (B10) . When expressed without the NPD, GFP was diffusely distributed and soluble in both [psi.sup(-)] and [PSI.sup(+)] cells (Fig. 3B). Thus, the coalescence of GFP in [PSI.sup(+)] strains depended on both the attached NPD and the presence of preexisting [PSI.sup(+)] elements.

The Sup35 NPD can induce [PSI.sup(+)] elements in [psi.sup(-)] cells (B6) . In our study, aggregates appeared in a small percentage of the copper-treated [psi.sup(-)] cells after 1 hour of induction (B16) . When plated onto media selective for nonsense suppression but not selective for the NPD-GFP plasmid, heritable [PSI.sup(+)] elements were detected in a similar small percentage of cells (B16) . When the NPD-GFP fusion protein was expressed in [psi.sup(-)] cells at a higher level or for a longer period, bright points of coalescence appeared in a larger fraction of the cells (Fig. 3A) (B16) , and a correspondingly larger fraction showed conversion to [PSI.sup(+)] (Fig. 2C). Intense fluorescent foci were maintained in mother and daughter cells for at least 4 hours after NPD-GFP expression was repressed. In contrast, when NPD-GFP was expressed at high levels in an HSP104 deletion strain, which cannot propagate [PSI.sup(+)], GFP coalescence was observed in only a few rare cells (B16) . Thus, NPD-GFP coalescence is a marker of the heritable prionlike state of Sup35.

Next we used NPD-GFP to visualize cryptic [PSI.sup(+)] elements in cells expressing mutant Hsp104 proteins. Cells expressing the K218T and K620T proteins exhibited the diffuse fluorescence characteristic of [psi.sup(-)] cells, but many also contained the intense foci characteristic of [PSI.sup(+)] cells (Fig. 3D). In contrast, intense foci were rarely observed in cells expressing the K218TK620T double mutant. Thus, although some Sup35 protein was insoluble in the latter (Fig. 1F), it did not efficiently nucleate the coalescence of newly synthesized NPD-GFP nor the reappearance of [PSI.sup(+)] (Fig. 2B).

Unique properties of Sup35 [PSI.sup(+)] aggregates. To further probe the relation between protein aggregation and prion inheritance, we monitored the behavior of another aggregation-prone GFP protein, a run-on translation product generated by mutation of the termination codon (B15) . This protein (GFP-t) was more variable in expression than NPD-GFP, accumulating in only a fraction of the cells. In these, its distribution varied widely: In groups of budding cells, some individuals exhibited diffuse fluorescence, whereas others showed intense concentrated foci (Fig 3C). Thus, unlike that of NPD-GFP, the distribution pattern of GFP-t was not inherited.

During the formation of aggregates, other amyloids and prions are thought to abandon most of their normal structure. Because GFP fluorescence depends on proper tertiary structure (B17) , some structure must be maintained in the prionlike foci of NPD-GFP. We do not yet know whether the COOH-terminal domain of Sup35 retains its tertiary structure during prion formation, but further experiments demonstrated that it strongly affected the behavior of the NPD.

To determine how Hsp104 affects the solubility of the NH.inf(2)-and COOH-terminal domains of Sup35, we expressed them separately in wild-type cells and in HSP104 deletion mutants (Fig. 4). In wild-type cells, each domain was present in the supernatant and pellet fractions after a 100,000g spin. In hsp104 deletion mutants, the distribution of the COOH-terminal domain was unchanged, but the NH.inf(2)-terminal domain was found only in the pellet. Apparently, the NH.inf(2)-terminal domain has an intrinsic ability to interact with Hsp104, and through this interaction to undergo a change in state that alters its solubility (B18) . The aggregates formed by the NPD alone, however, behaved like the amorphous aggregates of denatured proteins that accumulate after heat shock and remain insoluble in HSP104

deletion mutants (B12) . This contrasted with the behavior of the NPD in its normal context, attached to the COOH-terminus, where Hsp104 was actually required for aggregation (Fig. 1D) and, moreover, was required at an intermediate concentration. Thus, the COOH-terminal domain of Sup35 profoundly alters the properties of the NPD and the consequences of its interactions with Hsp104.

The prion hypothesis in yeast. Our data demonstrate that Sup35 undergoes a change in state when cells convert from [PSI.sup(+)] to [psi.sup(-)] and from [psi.sup(-)] to [PSI.sup(+)]. This change involves the disappearance and appearance of a unique heritable aggregate that rapidly captures newly synthesized proteins containing the Sup35 NPD and is governed by the **chaperone** Hsp104 (Fig. 5). The ability of preexisting [PSI.sup(+)] elements to alter the conformational fate of newly synthesized prion proteins provides direct physical support for the prion hypothesis of [PSI.sup(+)] inheritance (B2) .

Aggregation is a hallmark of the change in state associated with the conversion of mammalian PrP.sup(C) to PrP.sup(Sc) (B19) . The many correlations we observed between the insolubility of Sup35 and the presence of [PSI.sup(+)] demonstrate that aggregation is characteristic of yeast prions as well. However, three findings indicate that [PSI.sup(+)] is more than a simple consequence of protein aggregation. First, a substantial fraction of Sup35 remained insoluble in cells expressing the K218TK620T double mutant, yet this material did not efficiently seed the propagation of [PSI.sup(+)] nor the coalescence of NPD-GFP. Second, when GFP was induced to aggregate through a COOH-terminal extension, the aggregates it formed were not heritable. Third, the aggregates formed by the NPD of Sup35 alone were affected by an Hsp104 deletion in a different manner than were the [PSI.sup(+)] aggregates of wild-type Sup35. We suggest that the COOH-terminal domain affects the packing of the Sup35 aggregates in a manner that is essential to [PSI.sup(+)] propagation.

Genetic analysis of yeast prionlike elements and the application of GFP fusion protein technology provide a supplement to mammalian investigations that should speed our understanding of self-propagating changes in protein structure and may lead to new approaches for therapeutic intervention in neurodegenerative diseases. But this work, together with work on another such yeast element, [URE3] (B2) (B20) , has yet broader implications. The existence of prions-elements of inheritance arising from alternative protein conformations-in both mammals and yeast suggests that they are broadly distributed in nature. In the mammalian brain, a nonmitotic tissue, prions were revealed by their capacity to function as infectious agents; in yeast, they were revealed by their ability to produce heritable changes in phenotype. A wide variety of elusive epigenetic phenomena in other organisms may well prove to depend on the maintenance of alternative protein structures. Finally, because the inheritance of the yeast [PSI.sup(+)] elements depends on Hsp104, a **chaperone** induced by environmental stress, this phenomenon provides a plausible mechanism for the inheritance of an environmentally acquired characteristic.

Figure F1
Caption: Sup35 aggregates in [PSI.sup(+)] but not [psi.sup(-)] cells. (A) Sup35 and Sup45 accumulation in [PSI.sup(+)] and [psi.sup(-)] cells. Electrophoretically separated total cellular proteins were reacted with antibodies directed against Sup35 and Sup45 (B7) (B22) (B25) . (B) Solubility of Sup35 in mid-log phase cells. Proteins fractionated by centrifugation at 12,000 or 100,000g were electrophoretically separated and reacted with antibodies against Sup35 (B22) , ribosomal protein L3 (B25) , and members of the Hsp70 and Hsc70 family [monoclonal antibody (mAb) 7.10].

(C) Solubility of Sup35 in stationary-phase cells. Proteins were analyzed as in (B) and reacted with antibodies to Sup35 (B22) and Hsp104 (B12) . Hsp104 is more readily visualized in stationary-phase cells because Hsp104 concentrations are higher, but similar results were obtained in log-phase cells (B10) . (D) Sup35 is soluble in cells overexpressing Hsp104. [PSI.sup(+)] 74D-694 cells (PSI .sup(+)) were converted to [psi.sup(-)] (PSI .sup(-)) by transformation with a centromeric plasmid pYS104 carrying the wild-type HSP104 gene, which increases Hsp104 expression two-to threefold as compared with that of wild-type cells (B4) . Fractionated lysates were analyzed as in (B). (E) Sup35 is soluble in HSP104 deletion mutants ((Delta) 104). [PSI.sup(+)] 74D-694 cells (PSI .sup(+)) were converted to [psi.sup(-)] (PSI .sup(-)) by transformation with a construct [hsp104::Leu2] (B4) that abolishes Hsp104 expression by disrupting the chromosomal HSP104 gene. Fractionated lysates were analyzed as in (B). (F) Solubility of Sup35 in cells harboring cryptic [PSI.sup(+)] elements. [PSI.sup(+)] 74-D694 cells were converted to [psi.sup(-)] by transformation with centromeric plasmids encoding Hsp104 proteins with Lys to Thr substitutions in the first (K218T) or second (K620T) or both (K218TK620T) NBDs (B4) . The parental strain (PSI .sup(+)) and its isogenic [psi.sup(-)] derivative (PSI .sup(-)) are also shown. Immmunoblots were quantified with ImageQuant software (Molecular Dynamics).

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Figure F2
Caption: Analysis of [PSI.sup(+)] propagation by colony formation. (A) Read-through of nonsense codons in [PSI.sup(+)] cells detected by the suppression of nonsense mutations. In 74-D694 cells, the suppressible marker is ade 1-14 (UGA). [psi.sup(-)] cells (PSI .sup(-)) do not form colonies on adenine-deficient medium (-ade) and are red on YPD; [PSI.sup(+)] cells (PSI .sup(+)) form colonies on adenine-deficient medium and are white on YPD (B4) (B13) . (B) Cells expressing K218T or K620T Hsp104 mutations carry cryptic [PSI.sup(+)] elements. [PSI.sup(+)] cells analyzed in Fig. 1F were spotted onto plates deficient in uracil (-ura) or adenine (-ade) or both. Growth without uracil forces retention of the plasmid. Growth on adenine-deficient medium requires read-through of the ade 1-14 UGA codon and the reappearance of [PSI.sup(+)]; it occurs only when cells are allowed to lose the K218T or K602T expression plasmid. (C) Transient expression of NPD-GFP induces [PSI.sup(+)]. [psi.sup(-)] 74-D694 cells carrying the GRE-regulated NPD-GFP expression plasmid (B15) were treated with DOC (1 (mu) M) for 1 or 4 hours. Equal numbers of induced (+DOC) and uninduced control (con.) cells were spotted onto YPD and adenine-deficient medium (-ade). [psi.sup(-)] strains underwent conversion to [PSI.sup(+)] after as little as 1 hour of induction, and the extent of conversion increased over time. NPD-GFP did not influence colony formation by [PSI.sup(+)] 74-D694 cells (B16) .

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Figure F3
Caption: Visualization of protein aggregates with GFP. (A) Diffuse distribution of NPD-GFP in [psi.sup(-)] strains (PSI .sup(-)) and coalescence in [PSI.sup(+)] strains (PSI .sup(+)). Isogenic [psi.sup(-)] and [PSI.sup(+)] cells (74D-694) were transformed with a CUPI or GRE NPD-GFP expression plasmid (B15) . CuS0.inf(4) (50 (mu) M) or DOC (1 (mu) M) was added to log-phase cultures for 1 or 4 hours. Because our antibodies do not recognize Sup35 protein in its native state, we could not perform colocalization studies. (B) GFP is diffusely distributed in all cells. Analysis was as in (A), except that the GRE regulated plasmid encoded GFP

without the NPD domain. (C) The heritability of GFP fluorescence patterns in groups of budding cells. Analysis was as in (A), with plasmids encoding NPD-GFP, GFP-t, and GFP (B15) under the control of a CUP1 promoter. (D) Cryptic [PSI.sup(+)] elements visualized by NPD-GFP fluorescence. Analysis was as in (A), (CUP1-regulated NPD-GFP) in cells transformed with plasmids expressing mutant Hsp104 proteins (see Fig. 1F).

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Figure F4
Caption: Hsp104 influences the solubility of the Sup35 NH.inf(2)-terminal domain. [psi.sup(-)] cells (74-D694) with an intact (WT) or disrupted ((Delta)) chromosomal HSP104 gene transformed with high-copy-number plasmids (B6) encoding either the Sup35 NH.inf(2)-terminal (NH.inf(2)-term.) or COOH-terminal (COOH-term.) domain. Lysates were subjected to centrifugation at 100,000g. Total lysate (T), supernatant (S), and pellet (P) were analyzed as in Fig. 1, with a polyclonal antiserum to Sup35 and mAb 7.10. Immune complexes were visualized with **horseradish peroxidase** -conjugated protein A and ECL reagent (Amersham).

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Figure F5
Caption: A model for prion formation in yeast. 1: Newly synthesized Sup35 (white shapes at left) interacts with the **chaperone** Hsp104 (black ovals at center). 2: Hsp104 helps Sup35 achieve a protein-folding transition state that is required for prion formation but is inherently unstable. 3: In the absence of [PSI.sup(+)], Sup35 reverts to its normal functional state. 4: Preexisting [PSI.sup(+)] elements capture and stabilize transition-state conformers; Sup35 is sequestered from translation and unfaithful termi-nation leads to nonsense suppression. 5: Transient overexpression of Sup35 nucleates prions de novo because the high concentration of transition-state conformers increases the likelihood of stabilizing intermolecular interactions (B23) . 6: In the absence of Hsp104, the transition state is difficult to attain and prions cannot be perpetuated. 7: Overexpression of Hsp104 might disturb the equilibrium in several ways: Hsp104 might bind prion-state conformers and disaggregate them; rebind monomers, reducing their ability to be captured by [PSI.sup(+)] elements; or reduce the local concentration of transition-state conformers because they are dispersed in association with larger numbers of Hsp104 (B24) .

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7. Shown in Fig. 1A is Saccharomyces cerevisiae strain 74D-694 (MATa, ade1

his3 leu2 trp1 ura3) [PSI.sup(+)] and [psi.sup(-)] derivatives. Not shown is strain D1142-1A (B4) . ;

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9. The first dimension of the gel was performed with the Immobiline Dry Strip system (linear gradient, pH 4 to 7) and the second was performed on an ExcelGel SDS (gradient 8 to 18%) with a Multiphor II apparatus. For cell lysis (of strain 74D-694; [PSI.sup(+)] and [psi.sup(-)]), cells were grown on yeast extract, peptone, and dextrose (YPD) to 5 x 10.sup(6) cells per milliliter and electrophoresis was done according to the manufacturer's recommendations (Pharmacia Biotech). Total proteins were stained with Coomassie blue, transferred to Immobilon filters, and reacted with antibodies to Sup35 (peptide amino acids 137 to 151). Immune complexes were visualized as described in Fig. 4 (M. M. Patino and S. Lindquist, data not shown). ;

10. M. Patino and S. Lindquist, data not shown. ;

11. When cell lysates were treated with proteinase K, the Sup35 protein of [PSI.sup(+)] cells was more resistant to digestion than was that of [psi.sup(-)] cells, but unlike the case with PrP.sup(Sc),.sup(,) no specific protease-resistant fragments of Sup35 were detected (M. Patino, S. Lindquist, Y. Chernoff, unpublished data). ;

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13. In strain 74D-694, the [PSI.sup(+)].sup(-) suppressible marker is ade 1-14, a UGA codon. [PSI.sup(+)] cells produce white colonies on complete media (YPD) and grow without adenine supplementation; [psi.sup(-)] cells produce red colonies and require adenine. Cryptic [PSI.sup(+)] elements in 74D-694 cells expressing Hsp104 K218T or K620T proteins from URA vectors were detected by simultaneous platings on media (i) with adenine and without uracil (all cells grew), (ii) with neither uracil or adenine (maintenance of the plasmid abrogated [PSI.sup(+)]-mediated nonsense suppression and cells did not grow), and (iii) with uracil and without adenine (the small percentage of cells that had spontaneously lost the plasmid at the time of plating recovered [PSI.sup(+)]-mediated nonsense suppression and grew). Cells expressing the double mutant could not recover [PSI.sup(+)] and did not grow on medium with uracil and without adenine. ;

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15. To create GFP-t, a DNA segment encoding GFP but lacking the natural termination codon was amplified with primers 5 (prime) -CGGATCCCATATGAGTAAAGGAGAAGAACTT-3 (prime) and 5 (prime) -CTCGAGCTCACTAGTTTTGTATAGTTCATCATCCATGCC-3 (prime) . The polymerase chain reaction (PCR) product was subcloned into plasmid p2UGpd (pRS426) with GPD promoter (D. Nathan and S. Lindquist, unpublished data) as a Bam HI-Sac I fragment generating the plasmid p2UGFP-t. The complete open reading frame extends into the vector sequence and encodes an aggregation-prone GFP with a 46-residue COOH-terminal extension with the sequence TSELQLLFPLVRVNCALGVIMVIAVSCVKLLSAHNSTQHTSRKHKV-COOH (B21) . To create an unfused GFP, a termination codon was regenerated by cutting p2UGFP-t with Spe I, filling in the overhanging ends, and re-ligating (creating the plasmid p2UGFP). To create NPD-GFP, the DNA encoding the NPD of Sup35 was amplified from pEMBL-SUP2 (B6) with primers 5 (prime) -CGCGGATCCATGTCGGATTCAAACC-3 (prime) and 5 (prime) -CGCGGATCCATCGTTAACAACTTCG-3 (prime) and subcloned as a Bam HI fragment into p2UGFP. The resulting construct encodes the NH.inf(2)-terminal 253 residues of Sup35 fused to GFP. Each of these constructs (GFP-t, GFP, and NPD-GFP) was amplified by PCR and subcloned into p2UG [M. Schena, D.

Picard, K. R. Yamamoto, Methods Enzymol. 194, 389 (1991)], conferring DOC-inducible expression, and also into pCLUC [D. J. Thiele, Mol. Cell. Biol. 8, 745 (1988)] for copper-inducible expression. Fidelity of constructs was confirmed by dideoxy nucleoside triphosphate sequencing, and the mobility of the expressed proteins was determined by protein immunoblot analysis. ;
16. J.-J. Liu and S. Lindquist, data not shown. ;
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18. The ability of the NH.inf(2)-terminal domain to stimulate Hsp104 adenosine triphosphatase activity suggests that this interaction is direct (E. Schirmer and S. Lindquist, unpublished data). ;
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21. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. ;
22. Isogenic [PSI.sup(+)] and [psi.sup(-)] cells were grown to a density of ~5 x 10.sup(6) cells per milliliter in YPD. Cells were suspended in 50 mM tris-HCl (pH 7.5), 5 mM MgCl.inf(2), 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, cycloheximide (100 (mu) g/ml), 1 mM benzamidine, 2 mM phenylmethylsulfonyl, leupeptin (10 (mu) g/ml), pepstatin A (2 (mu) g/ml), and ribonuclease A (100 (mu) g/ml) and disrupted with glass beads at 4.Deg.C. Proteins resolved by SDS-polyacrylamide gel electrophoresis were transferred to Immobilon membranes (Millipore) and reacted with an antiserum against amino acids 137 to 151 of Sup35 or against Sup45 (gift of M. Tuite), followed by .sup(125)I-conjugated protein A (ICN Pharmaceuticals), and then exposed to a PhosphorImager screen (Molecular Dynamics). ;
23. These interactions may be facilitated by the simultaneous binding of several Sup35 proteins to an Hsp104 hexamer or by rapid sequential binding and release of individual conformers in its immediate vicinity. [PSI.sup(+)] is drawn as an ordered aggregate, with reference to a model for mammalian prion formation (B19) . [PSI.sup(+)] aggregates have special properties, but we do not yet know if they form an ordered structure. ;
24. In preliminary experiments, GFP-marked prions do not disaggregate rapidly when Hsp104 is overexpressed (J.-J. Liu, unpublished data). Given their size, this is not surprising. However, because the prion assay relies on colony formation, and because Hsp104 is long-lived, it is possible that overexpression of Hsp104 simply prevents new prion conformers from joining the prion while preexisting prions are diluted by cell division. ;
25. We thank Y. Chernoff, S. Liebman, I. Kerkatch, and M. F. Tuite for helpful discussions; M. F. Tuite for antibodies to Sup45; J. Warner for antibodies to ribosomal protein L3; M. D. Ter-Avananesyan and V. V. Kushnirov for plasmids and polyspecific Sup35 antisera; N. Patel for help with figures; and M. Singer, S. Rutherford, and S. K. DebBurman for comments on the manuscript. Supported by a grant from the National Institute of General Medical Sciences (NIH grant GM25874), and the Howard Hughes Medical Institute. J.-J.L. was supported by The Markey Program.
Descriptors: General Cell Biology

(THIS IS THE FULLTEXT)

...Abstract: green fluorescent protein fusion. Aggregation depended on the intracellular concentration and functional state of the **chaperone** protein Hsp104 in the same manner as did [PSI.sup(+)] inheritance. The amino-terminal and...

...Text: can induce de novo heritable [PSI.sup(+)] elements (B2) (B6) . Moreover, transient overexpression of the **chaperone** Hsp104 can restore translational fidelity, heritably converting cells from [PSI.sup(+)] to [psi.sup(-)] (B4...

...did immunostaining show any difference in the sedimentation of Sup45, ribosomal protein L3, or the **chaperone** proteins Hsp70, Hsp90, and Hsp104 (Fig. 1, B and C) (B10) . High salt (1 M...

...Role of the **chaperone** Hsp104 in Sup35 aggregation. Overexpression of Hsp104, a protein that promotes the resolubilization and reactivation... that rapidly captures newly synthesized proteins containing the Sup35 NPD and is governed by the **chaperone** Hsp104 (Fig. 5). The ability of preexisting [PSI.sup(+)] elements to alter the conformational fate... structures. Finally, because the inheritance of the yeast [PSI.sup(+)] elements depends on Hsp104, a **chaperone** induced by environmental stress, this phenomenon provides a plausible mechanism for the inheritance of an... with a polyclonal antiserum to Sup35 and mAb 7.10. Immune complexes were visualized with **horseradish peroxidase** -conjugated protein A and ECL reagent (Amersham...
...prion formation in yeast. 1: Newly synthesized Sup35 (white shapes at left) interacts with the **chaperone** Hsp104 (black ovals at center). 2: Hsp104 helps Sup35 achieve a protein-folding transition state...

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